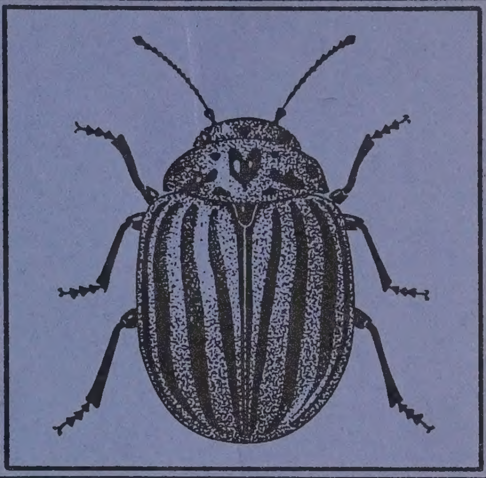
Gypsy moth, etc

F1 sterility
male trapping, etc

1985



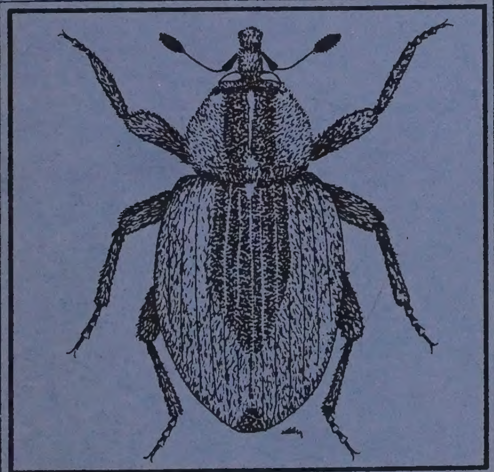
1985

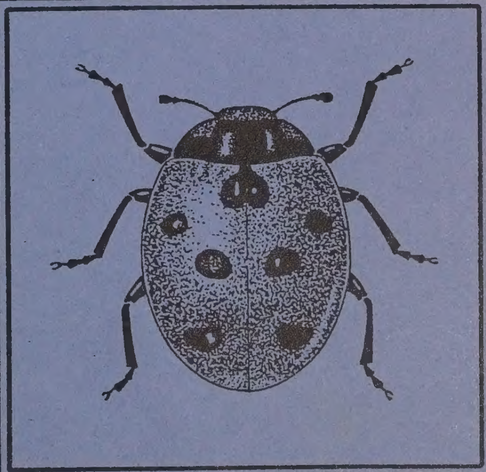
United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service





October 1, 1984 - September 30, 1985

Laboratory Report
Otis Methods Development Center
Animal and Plant Health Inspection Service
United States Department of Agriculture
Otis Air National Guard Base
Massachusetts
02542

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Laboratory Report
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Massachusetts
02542

Jessie J. Baker	Supervisory Biological Technician
Joyce A. Finney	Biological Technician
Othel T. Forrester	Entomologist
Billy G. Fulp	Electrician
Teri A. Hill	Office Clerk
Philip C. Kingsley	Entomologist
Victor C. Mastro	Entomologist
Winfred H. McLane	Biological Technician
Edward C. Paszek	Agriculturist
Charles P. Schwalbe	Center Director
John A. Tanner	Entomologist
Joseph G. R. Tardif	Equipment Specialist
Rebecca Upton	Secretary

Collaborators:

Juliet D. Tang	Research Associate
David R. Lance	Research Assistant

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Teri Hill for her
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SUMMARY

Several materials were tested in the field as candidate gypsy moth ovicides. Sun spray oil, soybean oil, Norpine, Scalecide and DX Insect Spray prevented hatch when applied to egg masses from November through April.

Extensive tests were done with several formulations of *Bacillus thuringiensis* (*Bt*) mixed with a variety of commercial stickers. Again, RA-1990, Bond, Plyac and Nufilm were shown effective to varying degrees. A new liquid polymer additive, Holdup, was tested extensively with Dipel 8L. Its incorporation into the formulation resulted in faster kill, greater mortality and better foliage protection than when Dipel 8L was sprayed alone. Numerous tests characterizing this additive were conducted. Other studies were done with a novel *Bt* encapsulation system developed at the University of Georgia. Tank samples recovered from the 1985 Lane County, Oregon Dipel 8L treatment were bioassayed. Other formulations of CME-13406, Dimilin, Maverik, UC-84572, UC-86874 and Methoxychlor-Diazinon were also assayed. Some tests were done to characterize feeding deterency of *Bt* formulations.

Case examples of successful use of *Bt* for eradication of isolated infestations are given. Reports from California, Illinois, Michigan, Minnisota, North Carolina, Tennessee, Washington and Wisconsin generally illustrate the types of non-chemical approaches being employed for eradication of isolated infestations.

1985 field tests with *Bacillus thuringiensis* concerned evaluation of the formulations: Dipel 8L, SAN 415, Thuricide 48LV, Biochem, Thuricide 24B and Thuricide powder. Because of erratic behavior of the populations in the test area, assessments based on egg mass counts are not possible. It was concluded, however, that the NRD-12 strain was somewhat more effective than HD-1.

Extensive tests have been conducted in the area of spray deposit assessment. Several droplet collection devices were evaluated at Moore Air Base, Mission Texas. Data are not yet analyzed but should give insight as to the relative effectiveness of various samplers in monitoring spray deposit.

Some field and laboratory tests were performed to assess the fate of gypsy moth egg masses buried in bark mulch piles, a scenario of interest from the regulatory standpoint. Results showed that the temperature in a mulch pile exceeds the minimum lethal temperature for egg masses. Also, ovicidal agents (chemicals) are present in bark mulch that devitalize eggs.

The utility of mass trapping in eradicating isolated gypsy moth infestations is discussed and the successful eradication of a population from Monona, Wisconsin described.

A project was conducted in Kent County, Maryland to determine the impact of releasing partially sterile male pupae and the extent to which sterility could be observed in the successive adult generation. While overflooding ratios were achieved in 1983 that should have resulted in large numbers of sub-sterile male x normal female matings, sampling in 1984 indicated that very few sterile F_1s were present. Possible explanations for this result will await further analysis of the data.

The dispersal and host-seeking behavior of newly hatched F_1 larvae was tested. Results are preliminary but indicate that F_1 larvae exhibit a deficiency in their ability to orient to vertical silhouettes nearby. More detailed behavioral assessments are underway.

The gypsy moth pheromone dispensers used in the 1983-1985 survey programs were tested; it was again shown that the field life of these dispensers is at least 12 weeks and that attractiveness changes somewhat during the course of the season. It was also shown that, in release recapture studies, moths tend to fly further from the point of release in the absence of pheromone sources.

The sterile F_1 demonstration projects in Bellingham, Washington and Darke County, Ohio are described in detail and initial estimates of overflooding ratios established. In Bellingham, we estimate that only seven wild females mated with wild males in 1985. Accordingly, additional releases are planned for 1986 to ensure eradication. In Ohio, all of the data suggest that the overflooding ratios achieved were adequate to effect eradication. Therefore, that site will only be monitored in 1986 to assess population status.

The gypsy moth rearing program is summarized. Experimental insects were sent to at least 15 cooperators. A stockpile of over 1,000,000 F_1 egg masses was established and over 76,000,000 colony eggs were produced in FY 1985. Quality control observations on the colony are documented. Innovations in the rearing operation are described, including the pupal conveyor system incorporated into the pupal harvesting facility, as well as other techniques for collecting and storing egg masses. Extensive tests were done to identify environmental conditions which permit producing eggs over a longer period. It was found that eggs held at high humidity can be embryonated longer than 28 days, thus, further opening the egg production window. Experiments were also done to determine the feasibility of incorporating strontium chloride into the parental diet with the aim of marking F_1 larvae. Results showed that strontium fades to background levels within 5 days of hatch. However, the technique shows great promise for marking newly hatched larvae for dispersal tests.

Further progress was made in facilitating the use of pheromones for detecting exotic pests. Various experiments were done with gypsy moth, concentrating on potential pheromone combinations and trap designs. In Australia, tests were done with the light brown apple moth and in India, the spiney bollworm. Tests in the Ivory Coast were conducted with the Egyptian cotton leafworm, false codling moth, nutgrass armyworm, pink bollworm and Old World bollworm. The guidelines for survey for fourteen exotic pests are presented, along with the results of pilot scale testing done in 1985 for *Autographa gamma*, *Chilo partellus*, *Chilo suppressalis*, *Epiphyas postvittana*, *Eupocillia ambiguella* and *Mamestra brassicae*.

The alfalfa weevil parasite redistribution program evaluation is presented; areas in the West are showing a continued increase in parasite establishment. *Bathyplectes anurus* appears to be displacing *B. curculionis*. *Microctonus aethiopoides* is also increasing in occurance in Missouri and Iowa and appears to be most important in impact on alfalfa weevil populations.

Rearing tests of alfalfa weevil are completed and a diet has been developed which is acceptable for use in a parasite recovery program.

The final Mexican bean beetle results from Ohio are inconclusive due mainly to the fact that MBB populations were very low in the years of the study.

Some tests were done to evaluate a new trap design for use against Japanese beetles at airports. The concept is to attract beetles to a station where they become contaminated with insecticide. These tests concerned the effectiveness of various insecticides in that context. Sevin XLR 5% EC appeared to be most effective.

Laboratory and field tests with the egg parasite of the Colorado potato beetle showed that it can be established in commercial situations; a test on Long Island successfully established Edovum puttleri in a plot under a spray schedule with SN-72129, a growth regulator. Various other tests were done in Massachusetts focusing on simplifying the methodology for monitoring field populations of Edovum puttleri. Various rearing tests were conducted, as well as methods for devitalizing and storing Colorado potato beetle eggs under refrigeration.

PESTICIDE TESTING SECTION

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PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

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Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

The main objective of this work is the development of new and improved treatments for regulated items moving from quarantined areas to non-infested locations. This project is primarily directed toward the development of treatments for recreational vehicles, mobile homes and outdoor household articles.

There is need for treatments to effectively control the hatch of gypsy moth eggs in regulatory situations. Compounds such as creosote have been used effectively in the past but may not be available in the future. Laboratory and field tests over the past 5 years have identified a number of materials that are effective when applied to egg masses. However, most materials that are efficacious in laboratory studies have proven to be ineffective in the field. Furthermore, agents must be effective regardless of the time of year (stage of the egg) they are applied.

During 1984-1985, seven materials (22 formulations) were field tested near Otis Air National Guard Base, Massachusetts. Starting in late November, 10 egg masses were treated with each material with a small hand sprayer. Egg masses were treated to the point of complete saturation. Treatments were made on a monthly schedule until April 15, 1985. Treated egg masses were left in the field until collected for hatchability tests in mid-April.

Egg collection was accomplished with 2 techniques. Five intact egg masses from each treatment date were collected into plastic petri dishes. Plugs of approximately 50 eggs each were removed from each of the 5 remaining masses. Untreated masses were collected at 2 locations within the study area for use as a check.

All egg masses were incubated in the laboratory at 27°C with 60% RH. After 10 days, percent hatch was estimated in dishes with complete masses. All eggs from each plug sample were counted and checked for hatch to give an accurate measurement of hatch.

Table 1. Percent hatch of gypsy moth egg masses field treated with various materials to study their effect on eggs throughout the diapause stage. Values are the average of 5 observations.

Material	Percent mix	Percent hatch as indicated by date treated					
		11/21	12/12	1/18	2/19	3/22	4/15
Sun Spray Oil	100	0	0	0	0	0	0
" + Iso Al	50	0	0	0	0	0	0
" + Iso Al	25	0	0	0	0	0	0
Soybean Oil	100	0	0	0	0	0	0
" + H_2O	50	0	0	0	0	0	0
" + Iso Al	25	0	0	0	0	0	0
Norpine	100	0	0	0	0	5	0
" + H_2O	50	0	3	0	0	0	0
" + H_2O	25	1	1	0	2	1	0
Citrus oil	100	87	90	80	93	65	90
" + H_2O	50	100	100	90	48	100	98
" + H_2O	25	100	100	100	95	70	0
Scalecide	100	0	0	0	0	0	0
" + H_2O	50	0	0	0	0	0	0
DX Insect Spray	100	0	0	0	0	0	0
" + H_2O	50	0	0		0	0	0
CRB-844	100	0	100	100	100	14	0
" + H_2O	50	0	100	80	100	60	4
Isopropyl Alcohol	100	100	100	100	100	100	95
Acetone	100	100	100	100	80	100	100
Kerosene	100	85	100	100	90	80	0
Control (West)	-	100	100	100	100	100	100
Control (East)	-	100	100	100	100	100	100

As in 1984 tests, soybean oil prevented hatch at all dosages throughout the study period. Norpine demonstrated for the second year that it will prevent nearly all hatch during the diapause stage. Sun spray oil, Scalecide and DX Insect Spray gave complete control of egg hatch throughout the study period.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

The objectives of this laboratory screening project are to collect and evaluate mortality data on experimental and registered compounds potentially useful for gypsy moth control, and to select materials for field studies and further development. These tests are designed to identify new materials and to increase the effectiveness of registered products.

Unless otherwise stated, all tests have been conducted with our standard red oak seedling technique. Test insects are of the New Jersey strain and have been laboratory reared on artificial diet.

Bacillus thuringiensis (Bt) was sprayed on over half of the total acreage treated for gypsy moth control in 1985. In isolated infestations, multiple applications of Bt was the principle treatment used. Post-spray trapping in and around treatment areas indicated good control was achieved. Single and multiple applications resulted in few, if any, problems with phytotoxicity or car finishes. All formulations mixed and handled well. Laboratory studies during this reporting period were directed mainly to the development of more effective Bt formulations.

Four stickers were tested with Dipel 8L (aqueous), Dipel 8L (oil) and Thuricide 48LV. RA-1990 was the most effective of the stickers tested followed by Bond, Plyac and NuFilm 17. When used with oil base Dipel, all stickers were more active after a 2 hour exposure to natural sunlight. With Dipel 8L (aqueous) only Plyac and NuFilm 17 were more effective after ultraviolet exposure. With Thruicide 48LV, stickers were not effected by natural sunlight.

NuLure did not enhance the feeding of 2nd instar gypsy moth larvae at the dosages tested. With higher dosages of NuLure, much less feeding occurred than on untreated checks. As the dosage dropped, feeding increased. Tender red oak seedlings were treated in a laboratory spray chamber with 5 replications per treatment. Some plants were then exposed to 2 hours of natural sunlight and then treated along with other plants to 0.25 inches of rainfall. Plants were then dried under a fan and exposed to 20 newly molted 2nd instar gypsy moth larvae per plant. Each test consisted of 2 checks, with and without sunlight. Mortality and defoliation readings were made after 2 and 4 days at 80^{O}F and 55% RH.

Table 1. Sticker tests with Dipel 8L (oil) at 12 BIU/96oz/acre.

							Mortality - Defoliation			
	No	No	No	Rain	UV		After 2 days		After 4 days	
Formulation	R	UV	S	.25"	2HR	STK	mor.	def.	mor.	def.
Dipel 8L	X	X	X				35	14	61	44
"	X		X		X		34	2	76	23
"		X	X	X			1	26	8	66
"			X	X	X		11	20	32	55
8L+2% RA-1990		X		X		X	10	26	29	56
"				X	X	X	24	9	50	38
8L+2% Bond		X		X		X	6	26	17	60
"				X	X	X	16	16	44	42
8l+2% Plyac		X		X		X	10	40	30	62
"				X	X	X	13	26	39	62
8L+2% NuFilm		X		X		X	1	38	9	78
"				X	X	X	7	22	19	60
Check	X	X	X				0	48	1	84
"	X		X		X		0	50	0	84

R = Rain
S = Sticker
STK = Sticker

Table 2. Sticker tests with Dipel 8L (aqueous) at 12 BIU/96oz/acre.

Formulation	No R	No UV	No S	Rain .25"	UV 2HR	STK	Mortality - Defoliation After 2 days mor.	def.	After 4 days mor.	def.
Dipel 8L	X	X	X				48	3	89	8
"	X		X		X		50	3	84	8
"		X	X	X			11	26	26	69
"			X	X	X		1	38	7	67
8L+2% RA-1990		X		X		X	44	6	80	18
"				X	X	X	36	8	84	15
8L+2% Bond		X		X		X	31	7	78	17
"				X	X	X	43	5	79	17
8L+2% Plyac		X		X		X	14	21	30	62
"				X	X	X	26	7	59	32
8L+2% NuFilm		X		X		X	5	28	13	87
"					X	X	14	17	47	54
Check	X	X	X				0	68	1	100
"	X		X		X		1	62	2	90

R = Rain
S = Sticker
STK = Sticker

Table 3. Sticker tests with Thuricide 48LV, at 12 BIU/96oz/acre.

Formulation	No R	No UV	No S	Rain .25"	UV 2HR	STK	Mortality - Defoliation After 2 days mor.	def.	After 4 days mor.	def.
Thuricide 48LV	X	X	X				17	3	79	8
"	X		X		X		28	3	75	8
"		X	X	X			14	2	71	10
"			X	X	X		9	3	60	11
48LV+ RA-1990		X		X		X	24	1	87	4
"				X	X	X	27	1	79	9
48LV+ Bond		X		X		X	17	1	81	5
"				X	X	X	14	1	88	3
48LV+ Plyac		X		X		X	8	4	52	18
"				X	X	X	10	3	61	25
48LV+ NuFilm		X		X		X	9	3	60	18
"					X	X	9	2	46	20
Check	X	X	X				0	78	0	100
"	X		X		X		0	76	0	90

R = Rain
S = Sticker
STK = Sticker

NuLure was tested to see if it would stimulate feeding of 2nd instar larvae. Oak discs approximately 1.0 inch in diameter were sprayed with various amounts of NuLure and NuLure + Bt using a small bottle sprayer. Treated discs were then placed on clips in a gallon container along with untreated foliage. Twenty larvae were then placed in each container and given the option of feeding on treated or untreated material. After 24 hours, an estimation of defoliation was made.

Table 4. Percent defoliation of red oak foliage discs treated with NuLure at various concentrations and exposed to 20 2nd instar gypsy moth larvae for 24 hours.

Treatment	Percent Defoliation			
	Rep. 1	Rep. 2	Rep. 3	Average
25% NuLure	75	90	60	75
Check (Untreated)	90	75	80	82
50% NuLure	40	40	50	43
Check (Untreated)	95	98	78	90
100% NuLure	15	30	20	22
Check (Untreated)	30	70	95	65

Table 5. Percent defoliation of red oak foliage discs treated with NuLure and Bt then exposed to 2nd instar gypsy moth larvae for 24 hours.

Treatment	Percent Defoliation			
	Rep. 1	Rep. 2	Rep. 3	Average
Dipel 8L (oil) + 33% NuLure	15	15	5	12
Dipel 8L (oil)	15	20	5	13
Dipel 8L (oil) + 16.5% NuLure	10	40	30	27
Dipel 8L (oil)	10	25	15	17
Dipel 8L (oil) + 8.25% NuLure	20	10	5	12
Dipel 8L (oil)	30	20	20	23
Thuricide 48LV + 33% NuLure	15	30	6	17
Thuricide 48LV	20	25	5	17
NuLure 33%	95	95	95	95
Check (Untreated)	98	98	95	97

Test results continue to demonstrate the effectiveness of RA-1990 and Bond when used with Bt. Natural sunlight did enhance the activity of all stickers when incorporated at 2% by volume with Dipel 8L (oil) and Dipel 8L (aqueous). Dipel 8L (oil), without sticker, with exposure to sunlight for 2 hours gave added weathering protection. Dipel 8L (aqueous) and Thuricide 48LV without stickers was not aided by sunlight. As expected, little Thuricide 48LV was washed off with 0.25 inches of rainfall. Based on this work and previous work, we would continue to rate the tested stickers based on effectiveness as: (1.) RA-1990, (2.) Bond, (3.) Plyac, and (4.) NuFilm 17.

Holdup, a liquid polymer system, was tested with Dipel 8L (oil). This combination resulted in faster kill, greater overall kill and greater foliage protection than when Dipel 8L (oil) was sprayed alone.

A combination of 1 part water and 1 part Holdup with Bt and Holdup and Bt only appeared to be the most effective formulations tested. Holdup 1128540-0 appeared to be slightly more effective than 1128540.

Applications of Holdup only onto oak seedlings had no toxic effect on 2nd instar gypsy moth larvae.

Table 6. Percent mortality of 2nd instar gypsy moth larvae with defoliation after a 2 and 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup.

		Percent Mortality - Defoliation			
	BIU	2 days		4 days	
Formulation	96 oz/acre	mor.	def.	mor.	def.
Dipel 8L	8	17	8	50	33
Dipel 8L	12	35	1	86	20
1ml 8L + 4ml H_2O + 1ml Holdup 1128540	8	18	12	47	45
1ml 8L + 4ml H_2O + 1ml Holdup 1128540-0	8	28	8	64	38
2ml 8L + 4ml H_2O + 2ml Holdup 1128540	12	39	4	88	6
2ml 8L + 4ml H_2O + 2ml Holdup 1128540-0	12	73	1	97	3
2ml 8L + 5ml H_2O + 1ml Holdup 1128540	12	52	2	89	8
2ml 8L + 5ml H_2O + 1ml Holdup 1128540-0	12	15	9	44	36
Check	-	0	100	0	100

Table 7. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 12 BIU/96 oz/acre.

Formulation	Inches rain	Percent Mortality	Percent Defoliation
Dipel 8L	-	57	27
Dipel 8L	0.5	9	82
2ml 8L + 3ml H_2O + 3ml Holdup 1128540	-	84	7
"	0.5	14	78
2ml 8L + 3ml H_2O + 3ml Holdup 1128540-0	-	84	17
"	0.5	9	78
Check	-	3	92

Table 8. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 12 BIU/96 oz/acre.

Formulation	Percent Mortality	Percent Defoliation
Dipel 8L	78	33
2ml 8L + 3ml H_2O + 3ml 1128540	96	7
2ml 8L + 5ml H_2O + 1ml 1128540	93	17
2ml 8L + 5.5ml H_2O + .5ml 1128540	79	31
2ml 8L + 5.8ml H_2O + .2ml 1128540	68	38
2ml 8L + 3ml H_2O + 3ml 1128540-0	87	20
2ml 8L + 5ml H_2O + 1ml 1128540-0	82	28
2ml 8L + 5.5ml H_2O + .5ml 1128540-0	75	39
2ml 8L + 5.8ml H_2O + .2ml 1128540-0	66	41
Check	1	100

Table 9. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 3 and 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 12 BIU/96 oz/acre

Formulation	Percent Mortality - Defoliation			
	3 days		4 days	
	mor.	def.	mor.	def.
Dipel 8L	41	25	59	40
2ml 8L + 6ml 1128540	56	9	86	10
2ml 8L + 3ml H_2O + 3ml 1128540	83	3	95	3
2ml 8L + 6ml 1128540-0	81	6	94	6
2ml 8L + 3ml H_2O + 3ml 1128540-0	74	6	97	6
Check	2	88	3	91

Table 10. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 2, 3 and 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 6 BIU/96 oz/acre.

Formulation	Percent					
	2 days		3 days		4 days	
	mor.	def.	mor.	def.	mor.	def.
Dipel 8L	0	32	1	50	25	60
1ml 8L + 7ml 1128540	38	15	56	17	73	19
1ml 8L + 3.5 ml H_2O + 3.5ml 1128540	24	13	41	17	66	24
1ml 8L + 7ml 1128540-0	54	5	77	5	91	5
1ml 8L + 3.5 ml H_2O + 3.5ml 1128540-0	28	16	37	27	47	46
Check	0	92	0	100	0	100

Table 11. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 2, 3 and 4 day exposure to oak seedlings treated with Dipel 8L (oil) and Dipel 8L (oil) with Holdup at 12 BIU/96 oz/acre.

Formulation	Percent					
	2 days		3 days		4 days	
	mor.	def.	mor.	def.	mor.	def.
Dipel 8L	13	8	36	20	65	38
2ml 8L + 6ml 1128540	31	8	53	10	74	10
2ml 8L + 3ml H_2O + 3ml 1128540	23	5	52	9	80	14
2ml 8L + 6ml 1128540-0	33	9	57	12	78	15
2ml 8L + 3ml H_2O + 3ml 1128540-0	46	4	71	6	88	8
Check	0	84	0	100	0	100

Table 12. Percent mortality of 2nd instar gypsy moth larvae and defoliation 5 days after exposure to oak seedlings treated with Holdup.

Formulation	Percent	
	Mortality	Defoliation
1 part H_2O + 1 part 1128540	0	96
1 part H_2O + 2 parts 1128540	0	96
1 part H_2O + 1 part 1128540-0	0	98
1 part H_2O + 2 parts 1128540-0	0	100
Check	1	100

San 415 (NRD-12) gave faster kill and less defoliation than standard HD-1 formulations of Bt. Mortality was higher with SAN-415 at all dosages tested.

Table 13. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to Bt treated foliage.

Formulation	Dosage BIU/96 oz/ac	Percent Mortality	Percent Defoliation
SAN 415	4	87	6
	6	78	5
	6*	82	7
	8	94	7
Thuricide 32LV	4	33	32
	6	20	26
	8	49	47
Thuricide 48LV	6	40	16
ABG 6163	4	65	13
	8	78	32
Dipel 4L	4	45	30
	8	61	41
Check	-	0	100

* Field sample

Although SAN-415 was affected by rainfall, the loss of activity was not as great as with Dipel formulations.

Table 14. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 4 day exposure to oak seedlings treated with <u>Bt</u> using 6 BIU/acre.

Formulation	Ounces Acre	Inches Rain	Percent Mortality	Percent Defoliation
SAN 415	96	-	98	3
	32	-	95	8
	96	.25	83	18
	32	.25	52	50
	96	.5	87	20
	32	.5	61	29
Dipel 4L	96	-	97	5
	32	-	77	28
	96	.25	2	100
	32	.25	24	81
	96	.5	13	76
Dipel 8L	96	-	9	84
	32	-	8	82
	96	-	3	100
	32	-	4	100
	96	-	1	100
	32	-	0	100
Check	-	-	0	100

Neat applications of Thuricide 24B (with sticker) were very effective in the laboratory. Undiluted treatments of Dipel were effective as long as they were not exposed to rainfall.

Table 15. Percent mortality of 2nd instar gypsy moth larvae and defoliation after a 4 day exposure to oak seedlings treated with neat applications of *Bt*.

Formulation	Dosage/Rate	Inches rain	Percent Mortality	Percent Defoliation
Thuricide 24B	12 BIU/64 oz/ac	-	91	7
		.25	76	19
		.5	85	9
		1.0	98	7
Thuricide 24B	18 BIU/96 oz/ac	-	97	2
		.25	98	3
		.5	96	5
		1.0	95	7
Dipel 4L	12 BIU/48 oz/ac	-	91	4
		.25	45	51
Dipel 8L	12 BIU/24 oz/ac	-	100	6
		.25	5	82
Check		-	0	100

Various formulations of Dipel were tested at a number of dosages, with and without stickers and exposure to rainfall. The Dipel 8L sample consistantly gave poor results.

Table 16. Percent mortality of 2nd instar gypsy moth larvae exposed to oak foliage treated with Dipel 8L with 3 dosages at 96 ounces of volume per acre.

Inches rain	Percent Mortality 4 BIU/acre	8 BIU/acre	12 BIU/acre
-	5	12	36
0.1	2	3	9
0.2	2	4	4
Check	0	0	0

Table 17. Percent mortality of 2nd instar gypsy moth larvae exposed to oak foliage treated with Dipel 8L at 12 BIU/96 oz/acre and exposed to 0.25 inches of rainfall.

Sticker	PERCENT MORTALITY AFTER 4 DAYS Percent Sticker Used 0.0	1.0	2.0	3.0
-*	28			
-	10			
Bond		5	15	6
Cheveron		6	6	2
Nufilm-17		8	4	10
Plyac		7	28	8
RA-1990		32	21	21
RA-2424		9	52	25
Check	0	0	0	0

* No rainfall

Table 18. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with Dipel 8L at 12 BIU/96 oz/acre and exposed to various amounts of rainfall.

PERCENT MORTALITY AFTER 4 DAYS								
Inches Rain								
0.0	0.1	0.2	0.25	0.3	0.4	0.5	0.75	1.0
31	8	9	7	3	7	1	10	0

Table 19. Percent mortality of 2nd instar gypsy moth larvae and defoliation of seedlings after a 4 day exposure to oak seedlings treated with various formulations of Dipel.

Formulation	Dosage BIU/96 oz	Percent Mortality	Percent Defoliation
Dipel 4L	8	83	13
	12	89	5
Dipel 6L	8	39	50
	12	28	54
Dipel 8L	8	70	12
	12	38	36
ABG-6165 Aqueous	8	83	22
	12	87	11
Check	--	0	100

Table 20. Percent mortality of 2nd instar gypsy moth larvae and defoliation of seedlings after a 7 day exposure to oak seedlings treated with Dipel 6L.

Dosage BIU/gal/acre	Percent Mortality	Percent Defoliation
6	90	5
12	96	2
24	98	0
Check	0	58

Table 21. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation 4 days after exposure to 3 dosages of Dipel 8L applied at 96 ounces per acre.

Dosage BIU/Acre	Inches rain	Percent	
		Mortality	Defoliation
8	-	44	42
8	0.25	4	80
12	-	75	14
12	0.25	39	64
16	-	92	8
16	0.25	9	76
Check	-	0	98

Table 22. Percent mortality of 3rd instar gypsy moth larvae and seedling defoliation 4 days after exposure to Thuricide 48LV and Larvo applied to oak seedlings at a rate of 96 ounces per acre.

Formulation	Dosage BIU/96 oz	Percent	
		Mortality	Defoliation
Thuricide 48LV	32	90	3
	24	90	2
	16	92	2
Larvo	32	0	88
	24	1	100
	16	1	100
Check	--	0	100

Table 23. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation 4 days after exposure to 3 Thuricide formulations applied to oak seedlings at 12 BIU/96 oz/acre.

Formulation	Inches rain	Percent Mortality	Percent Defoliation
Thuricide 48LV	--	64	13
	0.25	60	12
	0.5	56	18
Thuricide 48B	--	77	3
	0.25	84	5
	0.5	82	4
Thuricide 24B	--	67	10
	0.25	70	6
	0.5	74	6
Check	--	0	94

Thuricide 48LV was tested with a new encapsulant developed at the University of Georgia by Alvaro Villaneus and Dr. Himel.

Table 24. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to oak seedlings treated with Thuricide 48LV and encapsulant at 1 part Bt, 1 part encapsulant and 1 part water.

Dosage/Rate BIU/oz/acre	Encapsulant	Percent Mortality	Percent Defoliation
6 BIU/48 oz	-	67	6
	H1	59	6
	H2	63	9
	H4	65	9
12 BIU/96 oz	-	84	3
	H1	75	3
	H2	66	13
	H4	80	3
Check	-	0	100

Table 25. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to oak seedlings treated with Thuricide 48LV and encapsulant at 1 part Bt, 1 part encapsulant and 1 part water with exposure to natural sunlight.

Dosage/Rate BIU/oz/acre	Encapsulant	Hours sunlight	Percent Mortality	Percent Defoliation
12/96 oz	--	-	80	3
"	--	6.0	64	3
"	H1	-	79	3
"		6.0	93	1
"	H2	-	93	1
"		6.0	68	3
"	H4	-	89	1
"		6.0	50	2
Check	-	-	0	98
	-	6.0	0	98

Samples from the Rhode Island and Oregon Bt programs were bioassayed to confirm their efficacy against gypsy moth larvae.

Table 26. Percent mortality of 2nd instar gypsy moth larvae and defoliation of seedlings 4 days after treatment with Dipel 8L (Lot 76-533-BJ in Rhode Island).

Material	Percent 8 BIU/96 oz mor.	def.	12 BIU/96 oz mor.	def.	16 BIU/96 oz mor.	def.
533 Drum #1	34	42	35	55	85	15
533 Drum #2	38	51	75	38	86	18
Lab Standard	23	60	52	40	84	21
Check	0	100	0	100	0	100

Table 27. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after a 4 day exposure to oak seedlings treated with Dipel 8L (16 BIU/96oz./acre) samples from Oregon.

Lot Number	Trailer	Date received	Tank	Percent Mortality	Percent Defoliation
Lab Standard	-	-	-	96	11
76-556-BJ	6300	4-24-85	1	73	36
76-579-BJ	6991	4-24-85	2	91	11
Check	-	-	-	0	100
Lab Standard	-	-	-	80	13
76-553-BJ	7127	4-19-85	4	81	13
76-554-BJ	63172	4-21-85	3	84	6
Check	-	-	-	1	100
Lab Standard	-	-	-	80	16
76-585-BJ	6572	4-25-85	5	86	15
Check	-	-	-	1	100
Lab Standard	-	-	-	65	14
76-550-BJ	714	4-19-85	4	63	12
76-555-BJ	63059	4-21-85	3	51	20
76-559-BJ	8200	4-23-85	2	53	19
76-577-BJ	1343	4-20-85	4	38	24
76-582-BJ	63199	4-21-85	3	54	13
77-623-BJ	6712	5-10-85	4	48	25
77-663-BJ	6552	5-16-85	2	63	11
77-664-BJ	6550	5-17-85	2	51	10
Check	-	-	-	2	90
Lab Standard	-	-	-	71	17
76-557-BJ	6562	4-23-85	-	80	7
76-558-BJ	1616	4-23-85	1	88	7
76-580-BJ	6731	4-25-85	5	94	4
76-583-BJ	6783	4-25-85	1	87	9
76-5-BJ	6739	4-24-85	1	95	2
77-660-BJ	6925	5-15-85	2	90	7
77-661-BJ	6978	5-15-85	2	81	5
77-662-BJ	6995	5-16-85	2	89	5
Check	-	-	-	0	100
Lab Standard	-	-	-	76	14
76-552-BJ	4063	5-22-85	2	45	36
76-576-BJ	6580	4-23-85	2	62	14
77-637-BJ	62974	5-09-85	4	37	40
77-659-BJ	6579	5-14-85	4	83	10
Check	-	-	-	1	100
Lab Standard	-	-	-		
76-551-BJ	80984	4-19-85	4	70	16
76-578-BJ	62977	4-23-85	2	62	17
76-581-BJ	6996	4-26-85	5	89	4
77-658-BJ	9641	5-14-85	4	94	3
Check	-	-	-	2	98

Micrococcus pseudoflaicidifix was tested against 2nd instar gypsy moth larvae. The material was furnished by Dr. Joseph Concannon of St. Johns University. Tender oak seedlings were sprayed in a laboratory spray chamber with a series of dosages ranging from 59 to 295 million units per acre equivalents. Oak seedlings were also completely submersed in the stock solution and others were sprayed with a hand held sprayer. After test insects were introduced onto the oak seedlings, they were held in an environmental chamber for 15 days. This chamber was maintained at 26°C with 50% RH. Mortality readings were made on 4 different days over the 15 day period. After 3 days, oak seedlings were completely defoliated. At that time, larvae were transferred to artificial diet for the remainder of the test. No mortality occurred after 15 days post-spray with larvae exposed to seedlings treated in a spray chamber. Fourteen percent mortality occured 15 days after exposure to plants dipped in *Mp*. One percent mortality occurred as a result of the hand sprayer application. No mortality occurred in the checks.

Two growth regulators from Celamerck Gm6H & Company KG were tested on oak seedlings. Both materials were very efficacious at low dosages.

Table 28. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with insect growth regulators, CME-13406 and CME-13411 at a rate of 1.0 gallons per acre.

Dosage lbs. AI/acre	Inches rain	Percent Mortality CME-13406 after 4 days	Percent Mortality CME-13406 after 7 days	Percent Mortality CME-13411 after 4 days	Percent Mortality CME-13411 after 7 days
0.03	-	76	96	61	89
0.03	0.5	80	96	57	90
	1.0	83	94	75	96
0.06	-	91	96	84	98
0.06	0.5	90	99	84	96
	1.0	76	99	87	100
Check	-		0		
Check	0.5		1		
	1.0		0		

Table 29. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with insect growth regulators, CME-13406 and Dimilin at a rate of 1 gallon per acre.

Dosage lbs. AI/acre	Percent Mortality			
	CME-13406		Dimilin	
	after 4 days	after 6 days	after 4 days	after 6 days
0.06	75	97	81	96
0.03	65	99	82	96
0.015	70	93	75	96
0.0075	74	97	77	98
0.00375	65	97	81	100
0.0018	74	100	84	97
Check		0		0

Mavrik Aquaflow, a broad-spectrum fluvolinate insecticide, was tested against 2nd instar gypsy moth larvae.

Table 30. Percent mortality of gypsy moth larvae 1 and 4 days after exposure to oak seedlings treated with Mavrik.

Dosage lbs. AI/1.0 gal/Acre	Percent		
	Mortality		Defoliation
	after 1 day	after 4 days	after 4 days
0.25	47	88	1
0.125	13	74	2
0.0625	3	50	6
0.0312	1	25	9
0.0156	1	15	14
Check	0	0	100

Laboratory tests were conducted with 2 insect growth regulators from Union Carbide, UC-84572 and UC-86874. Dimilin was the test standard.

Table 31. Percent mortality of 2nd instar gypsy moth larvae after a 6 day exposure to plants treated with insect growth regulators.

Material	lbs. AI/gal/acre	Inches Rain	Percent Mortality
UC-84572	0.1	-	93
	"	1.0	92
	0.05	-	96
	"	1.0	94
	0.025	-	92
UC-86874	0.1	-	98
	"	1.0	96
	0.05	-	98
	"	1.0	80
	0.025	-	90
Dimilin	0.1	-	91
	0.05	-	94
"	0.025	-	97
Check	-	-	0

Laboratory testing was conducted with 3 Dimilin formulations. Dimilin 2 oil and Dimilin 4 aqueous formulations were compared with Dimilin 25 W/P.

Table 32. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with 3 formulations of Dimilin and exposed to rainfall.

		Percent Mortality Inches Rainfall			
Formulation	Dosage lbs/acre	0.0	0.1	2.0	4.0
Dimilin 25 W	0.03	94			
	0.06	100	100	100	100
Dimilin 4 Aqueous	0.03	100			
	0.06	100	100	100	100
Dimilin 2 Oil	0.03	98			
	0.06	95	100	100	100
Check		0	0	0	1

With increased environmental concerns about Carbaryl, a number of commercial applicators are changing to alternative materials. A combination of Methoxychlor-Diazinon is one alternative for application to gypsy moth infestations. Laboratory tests were conducted with this formulation at the request of Davey Tree Company.

Table 33. Percent mortality of 2nd instar gypsy moth larvae and seedling defoliation after exposure to oak seedlings treated with Methoxychlor-Diazinon and Sevin 80S applied at 1 gallon per acre.

			Percent			
			Mortality		Defoliation	
Material	Dosage lbs. AI/acre	Inches rain	after 1 day	after 3 days	after 1 day	after 3 days
Sevin 80 S	2.0	-	99	-	-	-
	1.0	-	99	-	-	-
		0.25	10	88	3	3
		0.5	6	65	10	28
		1.0	3	18	26	62
	0.5	-	87	-	-	-
		0.25	7	41	18	56
		0.5	8	46	20	54
		1.0	0	0	58	100
	0.25	-	86	-	-	-
		0.25	2	5	48	92
		0.5	0	0	64	90
		1.0	0	0	82	100
Methoxychlor Diazinon	2.0	-	99	-	-	-
	1.0	-	99	-	-	-
		0.25	41	100	1	1
		0.5	26	68	8	16
		1.0	9	37	11	25
	0.5	-	100	-	-	-
		0.25	11	63	3	18
		0.5	9	34	16	52
		1.0	7	36	10	24
	0.25	-	86	-	-	-
		0.25	0	11	32	68
		0.5	0	3	30	64
		1.0	0	0	56	100
Check	-	-	-	-	70	95

Dipel 8L was tested in the laboratory against 2nd instar browntail moth larvae. Because of spread and increased public awareness, limited ground treatments are now being considered by the Cape Cod National Seashore for control of browntail moth infestations. Bt is the only material being considered for a spray program.

Table 34. Percent mortality of browntail moth larvae and seedling defoliation 6 days after exposure to oak seedlings sprayed with Dipel 8L.

Dosage BIU/gal/acre	Percent Mortality	Percent Defoliation
64	87	1.2
32	86	1.3
24	89	1.4
20	93	0.8
16	96	0.7
12	86	1.6
8	80	2.4
4	45	9.0
Check	1	71.0

The field residual of a number of insecticide formulations was studied during the summer. Individual apple trees were sprayed with a gas powered back-pack sprayer to the point of run-off. Following treatment, foliage was collected at various times and returned to the laboratory for bioassay with 3rd instar gypsy moth larvae. Bioassays were discontinued after 42 days bacause of tough and turning foliage. Materials such as Sevin XLR and Dimilin continued to give excellent kill after 42 days. All materials were sprayed at the recommended label dosage. Poor results with Himel H5 (1x1) were due mainly to poor application resulting from an extremely viscous formulation.

In feeding tests with Thuricide 48LV and Dipel 8L, 1.0 inch oak foliage leaf discs were treated with various numbers of 2,000 micron size droplets and then exposed to 2nd instar gypsy moth larvae on filter paper in plastic petri dishes. Applications of Bt were made from a stock solution containing 12 BIU/96 ounces. Percent defoliation of each disc was recorded at various time intervals. All tests were replicated 5 times and included untreated controls.

Table 35. Percent defoliation of oak foliage discs treated with BT and exposed to 2nd instar gypsy moth larvae for various amounts of time.

Material	Larvae per Dish	No. Drops per Disc	Defoliation – Hours After Exposure 2	5	21	28	45	51	74	122
Thuricide 48LV	5	1	10	16	22	28	28	28	28	32
	5	2	7	16	22	26	26	26	26	26
	5	3	11	20	22	26	26	26	26	34
	1	1	3	6	6	9	13	15	15	15
	1	2	.6	6	6	6	6	6	6	6
	1	3	2	5	5	5	5	5	5	5
Dipel 8L	5	1	13	24	44	70	72	72	72	76
	5	2	6	12	28	34	34	34	34	40
	5	3	6	16	30	32	32	32	32	52
	1	1	1	4	14	27	42	44	50	52
	1	2	3	9	14	18	20	20	26	30
	1	3	2	7	16	20	20	20	20	22
Control	5	-	15	22	100					
	1	-	.4	6	48	72	94	98		

Table 36. Percent defoliation of oak foliage discs treated with Bt and exposed to 2nd instar gypsy moth larvae for various amounts of time.

Material	Percent of Disc Treated	Larvae per Disc	Defoliation Hours After Exposure 4 T	4 C	20 T	20 C	26 T	26 C	45 T	45 C	50 T	50 C
Dipel 8L	25	1	0	4	1	11	1	14	1	14	1	16
		5	0	3	1	13	1	19	1	21	1	23
	50	1	0	2	0	3	0	5	1	11	1	11
		5	0	5	1	9	1	13	1	17	1	17
	100	1	0	-	0	-	0	-	0	-	0	-
		5	1	-	1	-	1	-	1	-	1	-
	Circle around edge only	1	0	-	0	-	1	-	1	-	1	-
		5	0	-	1	-	1	-	2	-	2	-
Thuricide 48LV	25	1	0	3	0	6	0	8	0	12	0	16
		5	0	7	0	13	0	23	0	23	0	19
	50	1	0	3	0	4	0	4	0	6	0	6
		5	0	1	0	2	0	2	0	2	0	3
	100	1	0	-	0	--	0	--	0	--	0	--
		5	0	-	1	--	1	--	1	--	1	--
	Circle around edge only	1	0	-	0	--	0	--	0	--	0	--
		5	1	-	1	--	1	--	1	--	1	--
Control	--	1	-	3	-	34	48	70	80	--	--	--
	--	5	-	30	-	100	--	--	--	--	--	--

T = Treated
C = No Treatment

Gypsy moth larvae, regardless of size, avoid feeding on foliage that is treated with Bt. They will feed around Bt droplets on foliage and seldom do they actually consume a large portion of any one drop. Therefore, one would think that for best control in the field, application should be geared to producing as many droplets as possible on all target foliage. Droplets that are deposited onto target foliage should contain a lethal dosage of Bt.

Table 37. Percent mortality of 3rd instar gypsy moth larvae after a 4 day exposure to treated apple foliage collected from the field.

Material	Percent Mortality											
	Days After Treatment											
	2	3	7	10	14	18	22	25	29	36	39	42
Thuricide 48LV	92	87	90	73	23	27	40	23	33	47	23	0
Thuricide 48LV + H1(1x1)	92	90	90	67	93	53	97	90	100	87	73	7
Thuricide 48LV + H4(1x1)	87	80	80	83	83	37	93	83	100	90	47	7
Thuricide 48LV + H5(1x1)	43	--	70	47	67	73	27	--	80	17	--	--
Thuricide 48LV + H5(2x1)	93	--	100	30	73	77	23	--	67	10	--	--
Thuricide 48LV + H6(2x1)	83	--	93	33	73	73	53	--	53	3	--	--
Thuricide 48LV + 3% Bond	90	87	93	97	67	30	60	37	57	30	100	7
Dipel 8L	83	97	80	87	50	63	83	60	83	37	50	3
Dipel 8L + 3% Bond	95	97	100	97	100	63	93	100	100	77	47	20
Bactospeine	95	80	87	80	50	43	93	77	23	23	43	7
Sevin XLR	100	100	100	100	100	100	100	100	100	100	73	100
Dimilin	100	100	100	100	100	100	100	100	100	100	100	100
Check 1	18	0	10	13	3	0	10	3	0	27	0	0
Check 2	0	6	3	10	7	0	0	0	0	0	20	0
Check 3	0	--	7	0	3	13	0	--	0	0	--	--

Oak leaf discs were treated with a number of Holdup formulations to determine if the material has any activity as a feeding stimulant. Five discs of each formulation were offered to twenty 2nd instar gypsy moth larvae. After 18 hours, percent defoliation was computed. When Holdup was compared to untreated controls, dilutions, if any, were made with water.

Table 38. Average percent defoliation of untreated and Holdup treated oak foliage discs 17 hours after their exposure to 2nd instar gypsy moth larvae.

Material Comparison	Defoliation Treatment	Defoliation Untreated
Holdup 40 (Undiluted) - Control	68	58
Holdup 40 (1x1) - Control	94	70
Holdup 40 (1x2) - Control	90	72
Holdup 40 (1x3) - Control	66	76
Holdup 40 (1x4) - Control	80	84
Holdup 40 (1x5) - Control	76	62
Holdup 40-0 (Undiluted)-Control	50	92
Holdup 40-0 (1x1) - Control	80	66
Holdup 40-0 (1x2) - Control	84	86
Holdup 40-0 (1x3) - Control	80	56
Holdup 40-0 (1x4) - Control	74	62
Holdup 40-0 (1x5) - Control	88	76

Foliage treated with Holdup had slightly more defoliation after 17 hours, indicating that it may act as a mild feeding stimulus. Holdup 1128540 appeared to be somewhat more active than Holdup 1128540-0. There was no mortality of test insects recorded.

Table 39. Average percent defoliation of treated and untreated oak foliage discs 18 hours after their exposure to 2nd instar gypsy moth larvae.

Comparison		Defoliation	
Material I	Material II	I	II
Holdup 40 (2x1)	Untreated Control	58	70
Holdup 40 (3x1)	Untreated Control	62	72
Holdup 40 (4x1)	Untreated Control	70	72
Holdup 40 (5x1)	Untreated Control	70	68
Holdup 40-0 (2x1)	Untreated Control	68	66
Holdup 40-0 (3x1)	Untreated Control	68	74
Holdup 40-0 (4x1)	Untreated Control	70	86
Holdup 40-0 (5x1)	Untreated Control	50	72
Holdup 40 undiluted	Dipel 8L (undiluted)	15	1
Holdup 40 undiluted	Thuricide 48LV (undiluted)	23	0
Holdup 40 undiluted	Bactospeine (undiluted)	23	0
Holdup 40 undiluted	Dipel 8L (12 BIU/96 oz/sol)	38	0
Holdup 40 undiluted	Thuricide 48LV (12 BIU/96 oz/sol)	28	1
Holdup 40 undiluted	Bactospeine (12 BIU/96 oz/sol)	21	0
Holdup 40-0 undiluted	Dipel 8L (undiluted)	34	0
Holdup 40-0 undiluted	Thuricide 48LV (undiluted)	30	1
Holdup 40-0 undiluted	Bactospeine (undiluted)	22	0
Holdup 40-0 undiluted	Dipel 8L (12 BIU/96 oz/sol)	1	1
Holdup 40 undiluted	Thuricide 48LV (12 BIU/96 oz/sol)	3	1
Holdup 40 undiluted	Bactospeine (12 BIU/96 oz/sol)	20	0

Tests were conducted with a number of Bt formulations on oak foliage discs held in petri dishes with 2nd instar larvae. Some discs were completely covered with material and others were treated with various numbers of drops.

Table 40. Average percent defoliation of treated and untreated oak foliage discs 24 and 48 hours after their exposure to 2nd instar gypsy moth larvae.

Comparison		Defoliation			
Material I	Material II	24 Hours		48 Hours	
		I	II	I	II
Thuricide 48LV (12BIU/96 oz/sol)1/	Untreated Control	0	62	1	80
48LV + Holdup-40 (1x1)1/	Untreated Control	0	22	13	88
Dipel 8L (12 BIU/96 oz/sol)1/	Untreated Control	1	28	2	62
8L + Holdup-40 (1x1)1/	Untreated Control	0	76	0	90
48LV + Holdup-40 (1x1)1/	48LV (12 BIU/96 oz/sol)	0	0	0	0
8L + Holdup-40 (1x1)1/	8L (12 BIU/96 oz/sol)	0	3	0	4
48LV + Holdup-40 (1x1)1/	8L + Holdup-40 (1x1)	1	0	1	0
48LV (12 BIU/96 oz/sol)2/	Untreated Control	27	22	46	56
48LV (12 BIU/96 oz/sol)3/	Untreated Control	18	23	30	40
48LV (12 BIU/96 oz/sol)4/	Untreated Control	2	1	14	28
48LV + Holdup-40 (1x1)2/	Untreated Control	54	44	96	78
48LV + Holdup-40 (1x1)3/	Untreated Control	20	16	54	66
48LV + Holdup-40 (1x1)4/	Untreated Control	15	24	40	76
8L (12 BIU/96 oz/sol)2/	Untreated Control	23	62	69	88
8L (12 BIU/96 oz/sol)3/	Untreated Control	11	25	44	68
8L (12 BIU/96 oz/sol)4/	Untreated Control	9	48	26	90
8L + Holdup-40 (1x1)2/	Untreated Control	29	37	93	98
8L + Holdup-40 (1x1)3/	Untreated Control	14	26	72	100
8L + Holdup-40 (1x1)4/	Untreated Control	8	60	62	97

1/ Entire disc painted with test material.
2/ One droplet of 2,000 micron size per disc.
3/ Three droplets of 2,000 micron size per disc.
4/ Five droplets of 2,000 micron size per disc.

The feeding effects of gypsy moth larvae were studied after their exposure to partially treated red oak seedlings. Two of 4 oak leaves on each plant were treated with Bt. Larvae were then exposed to the plants for 48 hours and given their choice as to feeding on treated or untreated foliage. Some untreated plants were tested to see if there was any difference as to leaf preference. Unless otherwise noted, Bt was applied from a 12 BIU/96 oz/solution.

Table 41. Average percent defoliation of treated and untreated oak leaves after a 48 hour exposure to various instar gypsy moth larvae.

Comparison		Defoliation							
		II Instar		III Instar		IV Instar		V Instar	
Material I	Material II	I	II	I	II	I	II	I	II
Thuricide 48LV1/	Untreated Control	--	--	2	18	1	8	4	31
Dipel 8L1/	Untreated Control	--	--	2	28	1	28	2	21
Untreated Control	Untreated Control	--	--	95	95	100	100	100	100
Thuricide 48LV2/	Untreated Control	32	50	18	50	10	27	28	50
Dipel 8L2/	Untreated Control	55	73	26	56	17	40	40	57
Untreated Control	Untreated Control	100	100	100	100	97	93	100	100
Thuricide 48LV3/	Untreated Control	15	37	13	22	4	13	7	12
Dipel 8L3/	Untreated Control	62	58	13	22	17	32	13	25
Untreated Control	Untreated Control	97	97	97	80	93	90	100	100
Thuricide 48LV4/	Untreated Control	38	52	12	41	14	29	--	--
Dipel 8L4/	Untreated Control	38	53	35	55	33	55	--	--
Untreated Control	Untreated Control	62	72	100	100	96	92	--	--

1/ Material applied by brush.
2/ Material applied in spray tower.
3/ Plants and larvae held in large black cages.
4/ Material applied in spray tower at 6 BIU/96 oz/acre.

Table 42. Average percent defoliation of oak foliage discs treated with various droplets of 2500 microns in size and exposed to 2nd and 3rd instar gypsy moth larvae for different time intervals.

			Defoliation			
Material	Instar	No. Drops cm^2	After 24 Hrs.	After 48 Hrs.	After 72 Hrs.	After 96 Hrs.
Dipel 8L	II	1	20	40	48	52
		3	9	14	20	20
		5	3	4	7	8
	III	1	6	11	11	11
		3	6	7	11	13
		5	3	8	11	11
Thuricide 48LV	II	1	22	28	40	40
		3	24	28	30	32
		5	5	10	15	15
	III	1	11	28	30	34
		3	21	26	28	28
		5	7	9	12	12
Control	II	-	44	74	100	100
Control	III	-	46	88	100	100

Table 43. Average percent defoliation of oak foliage discs treated with various droplets of 2,000 microns in size and exposed to 2nd and 3rd instar gypsy moth larvae for different time intervals.

Material	Instar	No. Drops cm^2	Defoliation After 1 Hr.	After 2 Hrs.	After 4 Hrs.	After 6 Hrs.	After 8 Hrs.	After 24 Hrs.
Dipel 8L + Holdup-40 (1x1)	II	1	2	7	20	29	30	64
		3	1	15	24	30	34	56
		5	1	6	15	21	28	42
	III	1	1	4	19	28	32	50
		3	2	6	14	17	18	25
		5	1	5	8	16	20	23
Thuricide 48LV + Holdup-40 (1x1)	II	1	6	20	34	48	56	92
		3	3	14	25	38	42	68
		5	4	15	26	34	40	70
	III	1	3	8	23	35	42	69
		3	2	10	16	28	32	44
		5	3	12	19	29	32	36
Control	II	-	2	21	28	42	56	100
	III	-	1	9	20	32	38	76

Table 44. Average percent defoliation of Bt treated oak foliage discs after exposure times to 2nd and 3rd instar gypsy moth larvae.

Comparison				Defoliation					
				6 Hrs.			24 Hrs.		
Material I	Material II	Material III	No. Drops cm^2	I	II	III	I	II	III
Thuricide 48LV + Holdup-40(1x1)	Thuricide 48 LV	Control	1	48	12	42	92	54	100
			3	38	15	--	68	54	100
			5	34	13	--	70	40	100
" 1/			1	35	30	42	69	62	100
			3	28	26	--	44	54	100
			5	29	10	--	36	20	100
Dipel 8L + Holdup-40(1x1)	Dipel 8L	Control	1	29	24	42	64	50	100
			3	30	12	--	56	27	100
			5	21	3	--	42	16	100
" 1/			1	28	17	42	50	40	100
			3	17	3	--	25	16	100
			5	16	2	--	23	22	100

1/ Third instar gypsy moth larvae.

Some controversy continues over the status of contact toxicity with insect growth regulators such as Dimilin and Alsystin. Based on laboratory and field tests, we have always felt that the growth regulators are excellent contact insecticides. They do not give quick kill of insects such as synthetic pyrethroids, but mortality of gypsy moth larvae will take place at the first molt following contact. We have, over the years, exposed all instars of gypsy moth larvae to Dimilin and Alsystin treated surfaces for various amounts of time and then reared them through on artificial diet. With this technique, there is a possibility that larvae may spread the material that they have picked up topically onto the surface of the diet and then take it through the gut, resulting in stomach poison kill. To rule out this theory, newly molted gypsy moth larvae were treated with various amounts of Dimilin and Alsystin with a microliter syringe. With acetone as a diluent, the material was applied directly behind the head capsule of each individual test larva. Twenty were treated for each dosage. Treated larvae were held individually in small containers with diet and mortality readings made over a period of time. With this test technique, it would be nearly impossible for the diet to become contaminated and any resulting mortality would surely be the result of contact with the insecticide.

Table 45. Percent mortality of various instar gypsy moth larvae following a topical application of Dimilin 25W from stock solutions of 0.06 lbs. AI/gal. and 0.03 lbs. AI/gal.

Microliters per Larva	Instar	Mortality Days After Treatment					
		6D	12D	16D	20D	24D	28D
1.0	II	100	--	--	--	--	--
0.2	II	100	--	--	--	--	--
1.0	III	70	90	90	95	--	--
0.2	III	25	60	90	95	--	--
1.0	IV	45	100	--	--	--	--
0.2	IV	10	15	50	75	75	80
1.0	V	0	20	30	35	--	--
0.2	V	0	0	0	0	0	0
1.0 1/	II	---	--	--	--	--	--
0.2 1/	II	60	90	100	--	--	--
1.0 1/	III	95	100	--	--	--	--
0.2 1/	III	10	40	80	90	90	--
1.0 1/	IV	45	55	60	85	90	--
0.2 1/	IV	0	0	15	60	90	--
1.0 1/	V	5	15	30	40	--	--
0.2 1/	V	5	5	5	--	--	--

1/ Applied from 0.03 lbs. AI/gal stock.

Table 46. Percent mortality of various instar gypsy moth larvae following a topical application of Dimilin 25W from stock solutions of 0.015 lbs. AI/gal and 0.0075 lbs. AI/gal.

Microliters per larva	Instar	Mortality Days After Treatment 6D	12D	16D	20D	24D	28D
1.0	II	--	--	--	--	--	--
0.2	II	45	80	100	--	--	--
1.0	III	95	100	--	--	--	--
0.2	III	5	16	60	100	--	--
1.0	IV	30	55	70	90	100	--
0.2	IV	0	0	20	65	80	85
1.0	V	5	20	20	--	--	--
0.2	V	0	0	0	0	0	0
1.0 1/	II	--	--	--	--	--	--
0.2 1/	II	45	85	100	--	--	--
1.0 1/	III	60	75	95	100	--	--
0.2 1/	III	0	20	60	95	--	--
1.0 1/	IV	30	35	50	70	90	--
0.2 1/	IV	0	0	20	35	50	--
1.0 1/	V	0	5	5	15	--	--
0.2 1/	V	0	0	0	0	0	0

1/ Applied from 0.0075 lbs. AI/gal stock.

Table 47. Percent mortality of various instar gypsy moth larvae following a topical application of 4 dosages of Dimilin 25W applied at 1.0 microliters per larvae.

Stock lbs AI/gal.	Instar	Mortality Days After Treatment 6 days	12 days	16 days	20 days
0.00375	II	55	75	75	75
	III	15	55	85	--
	IV	5	---	15	--
	V	0	5	---	--
0.00187	II	65	100	---	--
	III	0	25	75	--
	IV	0	---	35	--
	V	0	0	0	0
0.000935	II	45	95	100	--
	III	5	20	55	--
	IV	0	---	15	--
	V	0	0	0	0
0.000467	II	5	80	100	0
	III	0	10	30	0
	IV	0	0	0	0
	V	0	0	0	0
Acetone Only	II	0	0	0	0
	III	0	0	0	5
	IV	5	5	5	10
	V	0	0	5	10
Untreated Control	II	0	0	0	0
	III	0	0	0	5
	IV	0	0	5	5
	V	0	5	10	10

Table 48. Percent mortality of various instar gypsy moth larvae following a topical application of Alsystin 25W from stock solutions of 0.06 lbs. AI/gal. and 0.03 lbs. AI/gal.

Microliters per Larva	Instar	Mortality Days After Treatment 6D	12D	16D	20D	24D	28D
1.0	II	100	--	--	--	--	--
0.2	II	100	--	--	--	--	--
1.0	III	40	100	--	--	--	--
0.2	III	30	80	95	95	--	--
1.0	IV	45	90	100	--	--	--
0.2	IV	20	35	50	80	95	--
1.0	V	0	25	--	--	--	--
0.2	V	0	5	--	--	--	--
1.0 1/	II	100	--	--	--	--	--
0.2 1/	II	100	--	--	--	--	--
1.0 1/	III	45	100	--	--	--	--
0.2 1/	III	35	85	100	--	--	--
1.0 1/	IV	45	80	95	95	--	--
0.2 1/	IV	20	40	40	70	90	95
1.0 1/	V	5	40	45	--	--	--
0.2 1/	V	0	0	--	--	--	--
Acetone Only	III	0	0	0	0	5	5
Untreated Control	III	0	0	0	0	0	0

1/ Applied from 0.03 lbs. AI/gal stock.

Table 49. Percent mortality of various instar gypsy moth larvae following a topical application of Alsystin 25W from stock solutions of 0.015 lbs. AI/gal and 0.0075 lbs. AI/gal.

Microliters per larva	Instar	Mortality Days After Treatment 6D	12D	16D	20D	24D	28D
1.0	II	100	--	--	--	--	--
0.2	II	90	95	100	--	--	--
1.0	III	40	90	100	--	--	--
0.2	III	10	60	85	95	100	--
1.0	IV	25	70	90	90	100	--
0.2	IV	0	0	45	80	90	95
1.0	V	0	30	35	--	--	--
0.2	V	0	1	--	--	--	--
1.0 1/	II	100	--	--	--	--	--
0.2 1/	II	70	95	100	--	--	--
1.0 1/	III	20	90	100	--	--	--
0.2 1/	III	0	25	65	90	100	--
1.0 1/	IV	25	40	60	85	100	--
0.2 1/	IV	0	0	20	70	75	75
1.0 1/	V	0	10	15	--	--	--
0.2 1/	V	0	0	0	--	--	--

1/ Applied from 0.0075 lbs. AI/gal stock.

Late instar larvae treated with Dimilin and Alsystin were reared through to adults. Heavy mortality occurred with 4th and 5th instar treated larvae as a result of deformed pupae. This series of tests strongly indicates that Dimilin and Alsystin are, indeed, excellent contact insecticides when applied to gypsy moth larvae of all sizes.

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The number of isolated gypsy moth infestations has increased over the past decade, largely due to increased movement of recreational vehicles and family moves. More efficient survey techniques have also heightened our awareness of these infestations.

In most cases, the treatment of choice for eradication of those infestations is now multiple applications of Bacillus thuringiensis; this was prompted by a successful Bt program at Santa Barbara, California, in 1982. Since 1982, multiple applications of Bt have been used to treat a number of isolated infestations. Some states have applied multiple Bt applications followed by mass trapping. By far, the largest infestation to be treated with Bt was in Oregon, in 1985, when 225,000 acres were sprayed with Dipel 8L, using 3 applications from Beecomist nozzles.

This report reviews some of the Bt treatments of isolated gypsy moth infestations and documents the steady progress that has been made in establishing it as a tool for eradication projects.

California:

The first male gypsy moth was caught in Alameda County, California in 1973. Dimilin and Sevin were applied in 1977, to control gypsy moth at San Jose, Santa Clara County. No male moths were captured in or around the treatment area the following summer. In 1981, applications of Sevin were made in San Juan Capistrano, Orange County, with ground equipment. No male moths were captured after treatment.

An eradication program, with 6 aerial applications of Bt over 15 square miles of Santa Barbara County, was conducted in 1982. In addition, 3 ground applications of Sevin were made to 173 properties in the core area of the infestation. Following treatment, a mass trapping program was utilized in addition to the normal detection traps. No male moths were captured. During 1983, 1984 and 1985, a number of small isolated spots were treated with Sevin by ground equipment. No male moths were captured in traps following treatment.

In 1984, an area of San Diego County was treated with Sevin and Bt, and follow-up trapping resulted in the capture of 1 male moth. A small area in Felton was treated with 5 ground applications of Bt in 1985. No male moths were captured in a post-spray trapping survey.

Illinois

In the spring of 1983, Plant Protection and Quarantine began efforts to eradicate five outlying gypsy moth infestations with Bt or combinations of Bt and mass trapping. Eradication activities at these infestations in Illinois are being conducted in cooperation with the following State and local governmental entities:

Illinois Department of Agriculture
City of Bensenville, Illinois
City of Downers Grove, Illinois
City of Naperville, Illinois
City of Wheaton, Illinois
City of Wood Dale, Illinois
Addison Township in DuPage County, Illinois
DuPage County Forest Preserve, Illinois
St. Charles Township in Kane County, Illinois

The technologies applied were:

1. Two aerial applications of 2 quarts (35 BIU) Bt plus 2.5 ounces of Plyac sticker and 2 quarts of water to equal 1 gallon per acre, followed by mass trapping at 3 traps per acre.

2. Three aerial applications of 2 quarts (35 BIU) Bt plus 2.5 ounces of Plyac sticker and 2 quarts of water to equal 1 gallon per acre, followed by 32 traps per square mile (for evaluation and delimitation).

Table 1. The 1981-1983 history of moth catches and control or eradication actions.

Location	Year	Acres Treated	Applications and Dosages (BIU)	Type Trapping 1/	Moths Caught
Naperville, IL	1981			Del	36
	1982 2/	60	2 x 8	Del	17
	1983	40	2 x 17.5	MT (200)	0
Wood Dale/ Bensenville, IL	1981			Del	1479
	1982 2/	500	2 x 8	Del	166
	1983	989	2 x 17.5	MT (1532)	13
Wheaton, IL	1981			Del	286
	1982 2/	300	2 x 8	MT (300)	103
	1983	230	2 x 17.5	MT (800)	5
Downers Grove, IL	1981			Del	73
	1982 3/	800	2 x 8	MT (800)	28
	1983	50	2 x 17.5	MT (367)	1
St. Charles, IL	1981			Det	5
	1982			Del	26
	1983	90	3 x 17.5	Del	1

1/ MT (acres) - number of acres mass trapped at 3 traps per acre.
Del - delimited at 32 traps per square mile
Det - detection survey at 1 trap per square mile

2/ Two aerial applications of Sevin-4 oil were aborted. Infestations were partially treated with Bt followed by mass trapping.

3/ Two aerial applications of Sevin-4 oil were aborted. Bt was applied over the identical area followed by mass trapping.

In 1983, efforts to eradicate five isolated infestations in Illinois with applications of Bt or a combination of Bt and mass trapping equalled or surpassed expectations. Conclusions are that (1) eradication has been accomplished at Naperville, (2) eradication has been accomplished or has progressed toward that objective at the other four infestation locations, and (3) unusually high environmental stresses (extreme winter cold) could have been placed upon the life stages of the gypsy moth during 1982-1983 which would have biased these results towards eradication. Subsequent work will determine whether these results, in general, can be duplicated.

Michigan:

Two applications of Bt, followed by an application of disparlure, were applied over 530 acres in Kent County, during 1981. The treatment area remained infested.

Minnesota:

Table 2. The 1983-1985 history of moth catches and control or eradication action.

Location	Year	Acres Treated	2 App. Bt 16 BIU/acre	Type Trapping[1]	Moths Caught	Egg Masses
Apple Valley	1984	--	--	DEL	219	124
(north)	1985	57	X	MT (117)	41	22
Apple Valley	1984	--	--	DEL	256	158
(south)	1985	51	X	MT (96)	47	3
Lakeville	1984	--	--	--	30	9
	1985	39	X	MT (100)	2	0
Sauk Rapids	1983	--	--	DEL	29	3
	1984	20	X	MT (3)	0	0
St. Anthony	1983	--	--	DEL	41	19
	1984	30	X	MT (121)	0	0
Stillwater	1983	--	--	DEL	6	0
	1984	32	X	MT (70)	0	0
White Bear Lake	1984	--	--	DEL	15	0
	1985	39	X	MT (105)	0	0

1/ MT (acres) - Number of acres mass trapped at 3 traps per acre.
DEL - Delimitated at 32 traps per square mile.

North Carolina:

An area of Wake County (225 acres) was sprayed with 2 applications of Bt in 1982. Based on post-spray male moth catches, the gypsy moth was eradicated in the treatment area. During 1984, Thuricide 48LV was applied in 2 applications on 611 acres. Dosage was 16 BIU/gal./acre. Applications were made with Hugh 500 and Jet Ranger helicopters. In 1985, Dipel 8L (16 BIU/gal./acre) was applied to 2,090 acres, with 2 applications. The sticker, Plyac, was mixed at 2% by volume. Applications were made with a Twin Beech aircraft equipped with flat fan nozzles (8020).

Oregon:

Oregon trapped its first gypsy moth in 1979. By 1981, increased trap densities had revealed a major infestation in Salem, where 1014 moths were trapped. In the summer of 1982, a 5,000 acre section of the city was treated by air with carbaryl. During the post-treatment monitoring, 212 moths were trapped in Salem; all but 7 were outside the treatment area. Moths were also detected in five new areas of the state.

In 1983, four sites had repeated finds. A season total of 227 moths were trapped. Positive areas were Ashland (3), Gresham (3), Corvalis (2), West Portland (53) and Salem (147). Carbaryl was applied over 50 acres in the Salem area.

In 1984, 11,660 acres were treated with Dipel 8L, at 16 BIU/96 oz./acre. Three applications were made by helicopter with flat fan nozzles.

Three applications of Dipel 8L were applied to 225,000 acres in 1985, at 16 BIU/96 oz./acre. The material was applied by helicopter with Beecomist nozzles. Major population reduction was observed following treatment and progress is being made toward eradication of this large infestation.

Tennessee:

Applications of Dimilin and Thuricide 48LV were made in 1984. Dimilin was applied twice to 12,320 acres and Bt to 1,178 acres, with 2 applications at 16 BIU/gal/acre. Plyac was mixed at 2% by volume. The materials were applied by 2 Hugh 500's and a Bell Jet Ranger helicopter, equipped with flat fan nozzles. Applications were made in Johnson County.

In 1985, Dipel 8L was applied twice to 3,796 acres in Johnson County, at 16 BIU/gal./acre. Plyac was incorporated into the formulation at 2% by volume. Dimilin was also applied in 2 applications to 47,998 acres. One DC3 and 2 Twin Beech aircraft applied all materials. Flat fan (No. 8020) nozzles were utilized for dispensing the materials. No male moths were captured in a post-spray trapping survey of the treatment area.

Washington:

In Washington, moths were trapped in the Bryn Mawr area of King County, in 1973. Discovery of 3 egg masses led to an eradication program in 1979, spraying Orthene with ground equipment over 400 acres. No moths were trapped inside the treatment area that year, but two moths were trapped at new sites in the county. In 1980, an increased trapping program resulted in the detection of moths in nine areas of King County and two areas of Clark County. Statewide, 175 moths were trapped; none were from the Bryn Mawr eradication area.

In 1981, Washington initiated eradication/suppression programs at four sites: Clearview, Felida, Ravenna Park and Lincoln Park. Each area utilized high density trap deployments (1800 traps/mi^2), combined with a ground treatment of the pesticide Orthene, in Clearview and Felida, while Bt, Dipel was applied to Ravenna Park and Lincoln Park. Post-treatment surveys yielded nine moths in Felida, four in Clearview, three in Lincoln Park and 223 in Ravenna Park. It should be noted that treatment was made only with permission of the owner, resulting in a substantial number of untreated properties. Season trap totals were 268 moths with eleven new areas recording activity. Egg masses were located in each of the treatment areas.

Eradication/suppression efforts continued in 1982, in the four targeted areas. High density trapping programs were repeated, with Orthene applied in Felida and Clearview. Post-treatment inspections located egg masses in three areas: Ravenna Park, Felida and Tacoma. No egg masses were located in Clearview. The moths trapped statewide, totaled 824 from 22 locations in six counties.

In 1983, Bt (Dipel 4L - 3 applications) was applied aerially over 2,520 acres in Ravenna Park, Felida and Tacoma. Plyac was mixed at 2% by volume. High density trapping was used in Ravenna Park only. Moth catches were sharply reduced in all areas; no moths were detected in Felida. However, in the Stellacoom-Phillips area, adjacent to the Tacoma treatment, 1,083 moths were trapped, suggesting that the necessary area of application had been underestimated. The seasonal total of adult moth trap catches increased to 1,260 in 18 locations.

Three applications of Dipel 8L were applied to 7,920 acres in 1984. The material was applied at 16 BIU/96 oz./acre with 2% Plyac as a sticker. Applications were made by helicopter equipped with 8004 flat fan nozzles. Twenty properties were treated twice with Orthene 75S, using ground equipment.

In 1985, no applications were applied by air. There was a need to treat only one property with 2 applications of Orhtene 75S with ground equipment.

Wisconsin:

Table 3. The 1981 - 1983 history of moth catches and control or eradication action.

Location	Year	Acres Treated	Applications and Dosages (BIU)	Type Trapping1/	Moths Caught
Elm Grove, WI	1981			Det	2
	1982			Del	70
	1983	60	2 x 17.5	MT (110)	5

1/ MT (acres) - number of acres mass trapped at 3 traps per acre.
DEL - delimitated at 32 traps per square mile.
DET - detection survey at 1 trap per square mile

Based on results of eradication projects with Bt in various states, it is clear that the material can be used effectively as an eradication tool. However, it is obvious that multiple applications at high dosages are needed and mass trapping may be an important component in achieving eradication.

Project Number: GM 5.1.1
Project Title: Field Studies with Bacillus thuringiensis
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: W. H. McLane, J. A. Finney and T. Roland

A new Bacillus thuringiensis strain (NRD-12) was compared with the standard (HD-1) strain. Laboratory tests continue to demonstrate increased activity with NRD-12. Testing was conducted with NRD-12 formulated by Zoecon and Biochem.

Studies were conducted to determine efficiency of 3 droplet sizes dispersed by aircraft. Dipel 8L was sprayed at 8 BIU/96 oz/acre. Droplet sizes tested were 100-400-600 micron, VMD. Neat (undiluted) applications of Dipel 8L and Thuricide 24B were tested and compared to diluted formulations. Also a Thuricide powder was mixed in a nurse tank and applied to forest land by aircraft.

Methods and Techniques

Table 1. Bacillus thuringiensis applied to 50 acre woodland plots during May, 1985, by fixed wing aircraft.

Material	Dosage BIU/acre	Rate oz./acre	Droplet size (VMD)
Dipel 8L	8	96	100
Dipel 8L	8	96	400
Dipel 8L	8	96	600
San-415 (NRD-12)	6	96	100
Thuricide 48LV	6	96	100
Biochem (NRD-12)	6	96	100
Dipel 8L	12	24 (neat)	100
Thuricide 24B	12	64 (neat)	100
Thuricide 24B	16	85 (neat)	100
Thuricide 48LV	12	96	100
Thuricide 48LV	16	96	100
Thuricide Powder	12	96	100
CHECK			

Fifty acre treatment plots were established on state lands in Burrillville and Scituate, Rhode Island. Experimental plots were also treated in Douglas State Forest, Douglas, Massachusetts.

Using a compass, topographical maps, rope and surveyor tape, plots were established and allocated to the treatments listed in Table 1. Boundary lines were surveyed and marked in flourescent orange tape and each corner tree was marked with double flourescent orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. Plots were located so that there would be a maximum number of corners on or near roadways.

Treatment evaluation consisted of pre- and post-spray egg mass counts, egg hatchability tests, post spray larval counts under burlap, defoliation observations and frass weighings and counts.

Within the center 10 acres of each plot, 20 prism points were established, 5 points on 4 parallel lines. During March and early April, pre-spray egg mass counts were made at each prism point. New egg masses were counted and recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 80-90 percent.

Burlap was placed on 10 oak trees at random in the center 10 acres of some plots. Counts were made on the number of gypsy moth larvae under each band following treatment. After each reading, all larvae were removed from under the burlaps. Readings continued until the start of pupation.

Six, 8 inch funnels were utilized in the center of some plots to collect frass following treatment. Funnels were located on stakes approximately 3 feet above the ground. Each funnel had a small plastic tube with screen end attached for collecting the frass. This technique worked well as long as there was no rain. Collection was limited to 24 hour periods of dry weather. Frass was dried and then weighed with individual pellets being counted. Frass collection continued until pupation had started.

At peak defoliation time (early July) a survey was conducted from the ground and by air. Total defoliation of all oak species was estimated at each prism point within each experimental plot. Aerial photographs were also taken of each spray plot and all checks.

For a more effective evaluation of droplet sizes white "Kromekote" cards were placed in one plot of each droplet size. At each site, cards were placed in the open and down through the tree canopy at 10 foot intervals. A line was attached through a screw eye at the very top of a tree and extended to the ground. Cards were placed along the line in the center area of the tree crown parallel to the trunk. Similar vertical card lines were erected in the open by running a line from one tree to another with a line down in the center open area. Cards were arranged in both a vertical and horizontal position along the line. Cards were placed onto the lines just prior to treatment and were removed 2 hours following application. Cards were evaluated with a Quantimat 900 at the University of California, Davis. Dye was mixed with the Dipel 8L formulation to better evaluate spray deposit.

Applications of Bt were made with a Cessna-Ag-truck aircraft equipped with conventional spray boom and flat fan nozzles. For applications requiring a droplet VMD of 100 microns, 8003 nozzle tips were pointed 45 degrees into the slip stream. To produce a VMD of 400 microns, 8008 nozzle tips were pointed 45 degrees aft. Nozzle tips 8015 pointed 45 degrees aft produced droplets with a VMD of 600 microns.

The aircraft was equipped with a 50-mesh in-line screen and quick drain valves. All nozzles utilized 50-mesh screens and the material was applied at 120 MPH in a 75 foot swath width, 50 feet above the tree tops.

All mixing was done in a nurse tank and material was then pumped into the aircraft. Water was first added to the nurse tank, then Bt and sticker. Mixes were blended by agitation for at least 10 minutes before being loaded into the aircraft. The nurse tank was equipped with a 50-mesh, in-line screen. All mixing and aircraft operation were centered at the Danielson, Connecticut, State Airport.

Spraying started on May 22, 1985 and was completed on May 25, 1985. At the start of the program, 80% of the gypsy moth larvae were 2nd instar, 19% were 1st instar with the remaining being early 3rd. Foliage, in general, was extended to approximately 40 percent.

Table 2. Characteristics of spray equipment and experimental Bt applications.

Material	Dosage BIU/acre	Rate oz/acre	Droplet size (VMD)	Size nozzle	Number nozzles
Dipel 8L	8	96	100	8003	45
Dipel 8L	8	96	400	8008	17
Dipel 8L	8	96	600	8015	8
San-415 (NRD-12)	6	96	100	8003	45
Thuricide 48LV	6	96	100	8003	45
Biochem (NRD-12)	6	96	100	8003	45
Dipel 8L	12	24 (neat)	100	8003	10
Thuricide 24B	12	64 (neat)	100	8003	25
Thuricide 24B	16	85 (neat)	100	8003	33
Thuricide 48LV	12	96	100	8003	45
Thuricide 48LV	16	96	100	8003	45
Thuricide Powder	12	96	100	8003	45
CHECK	--	--	---	----	--

Results

Table 3. Total cumulative measurements of frass and larvae based on 9 twenty-four hour readings following treatment.

Material	Total burlap count	Total frass weight	Number frass pellets
Dipel 8L (100 VMD) 1/	393	6.47 g	277
Dipel 8L (400 VMD) 1/	311	4.30 g	198
Dipel 8L (600 VMD) 1/	349	4.47 g	220
San 415 (NRD-12) 2/	813	2.56 g	268
Biochem (NRD-12) 3/	124	.66 g	128
Thuricide 48LV 2/	331	2.92 g	314
CHECK 1/	708	5.85 g	306

Figures represent the total of all replications for that treatment.

1/ Replicated 4 times
2/ Replicated 3 times
3/ Replicated 2 times

Table 4. Percent population change based on pre- and post-egg mass counts and defoliation for each treatment.

Material	Percent defoliation	Pre-Spray EM/acre	Post-Spray EM/acre	Percent change
Dipel 8L (100 VMD)	23	3161	1903	- 40
Dipel 8L (400 VMD)	16	3159	1908	- 40
Dipel 8L (600 VMD)	27	3121	3043	- 2
Dipel 8L (neat) 1/	12	1455	1331	- 9
San-415 (NRD-12)	38	2020	2187	+ 8
Biochem (NRD-12)	5	118	41	- 65
Thuricide 48LV	21	1355	2871	+ 53
CHECK	37	2729	2596	- 5
Thuricide 24B 2/	57	3803	848	- 78
Thuricide 24B 3/	38	4951	1032	- 79
Thuricide 48LV 4/	33	3688	1488	- 60
Thuricide 48LV 5/	48	5641	1110	- 80
CHECK	90	5568	1267	- 77
Thuricide Powder	12	1260	1655	+ 24
CHECK	38	1005	3520	+ 71

1/ Applied neat at 24 ounces per acre
2/ Applied neat at 64 ounces per acre
3/ Applied neat at 85 ounces per acre
4/ Applied diluted at 12 BIU/96 ounces/acre
5/ Applied diluted at 16 BIU/96 ounces/acre

Table 5. Dipel 8L droplet characteristics as supplied by a Quantimat 900 at Davis, California.

Material	Plot	Droplet VMD wanted	Nozzle	Actual Results VMD	Actual Results Droplets CM^2	Actual Results oz/acre
Dipel 8L (8 BIU/96 oz/ac)	22	100	8003	230	0.5	1.2
Dipel 8L (8 BIU/96 oz/ac)	3	400	8008	261	2.0	10.8
Dipel 8L (8 BIU/96 oz/ac)	1	600	8015	172	0.5	1.2

Dipel 8L applied undiluted at 24 ounces per acre provided good foliage protection but had little effect on population reduction based on egg mass density. This apparently was due to larvae that fed less but survived to produce new egg masses. Based on population reduction, the neat application with 12 BIU/acre was not nearly as effective as an application of 8 BIU/96 ounces/acre. As expected, the treatment was certainly more effective than diluted Thuricide 48LV applied at 6 BIU/96 ounces/acre.

Based on frass weighings and counts, larval counts under burlaps and defoliation, it would appear that a droplet size of approximately 400 microns (VMD) (261 microns VMD - Quantimat 900) is more effective than droplets of lesser size. This may be due to the fact that small droplets do not reach their target due to drift and/or evaporation and/or less potency per small droplet. Larger droplets may produce fewer droplets per leaf and larvae are able to feed around them. Larger droplets were found mainly in the top part of the tree with small ones found throughout the sampling area from the ground to the top. There were more medium size droplets (261 microns) per cm^2 than small ones, indicating that small ones never reached their target.

Data from the Quantimat 900 at Davis, California, indicate nearly twice as much material on the horizontal cards as on the vertical ones. These data state that we did not come close to producing the actual droplet sizes wanted. Regardless of the droplet size, we got little of the material to the target.

The NRD-12 strain of Bt was somewhat more effective than the standard HD-1 strain. Biochem NRD-12 was the most effective of the 2 formulations tested. However, populations were very low in plots treated with that material. The standard Thuricide 48LV treatment at 6 BIU/96 oz./acre performed very poorly.

Treatments of undiluted Thuricide 24B performed no better than the standard diluted Thuricide 48LV. A higher dosage of Thuricide 24B resulted in more effective foliage protection, but no additional population reduction.

After spraying out approximately 100 gallons of Thuricide 24B, we started to see buildup of material on nozzle tips. This could result in clogged nozzles on an operational program.

In general, all Bt treatments gave discouraging results. In some cases, foliage protection was achieved but little population reduction occurred. We are unable to make any recommendations based on the field work that was conducted. If this type of work is to continue with Bt, it must be directed at developing a major breakthrough that will completely alter its present efficiency. This might be in the form of a new strain (genetic engineering), better formulations, improved application techniques, feeding stimulants, adjuvants and encapsulants.

Project Number: GM 5.1.2
Project Title: Spray Deposit Assessment
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: W. H. McLane, J. A. Finney, T. Roland, W. Yendol and R. Reardon

For years, Kromekote spray cards (4x5 in.) have been used for the characterization of spray deposit. In recent years, water and oil sensitive cards have been developed. For checking swath width, droplet size and density, cards are put out at one end of a runway or open area in a horizontal position. Cards are placed 5 feet apart in a line approximately 300 feet long, depending upon the aircraft being checked. To determine if a spray area is, indeed sprayed, cards are placed on the ground in open areas within the treatment plot. If there is spray deposit on the cards, users eyeball droplet size and assume the target area is covered.

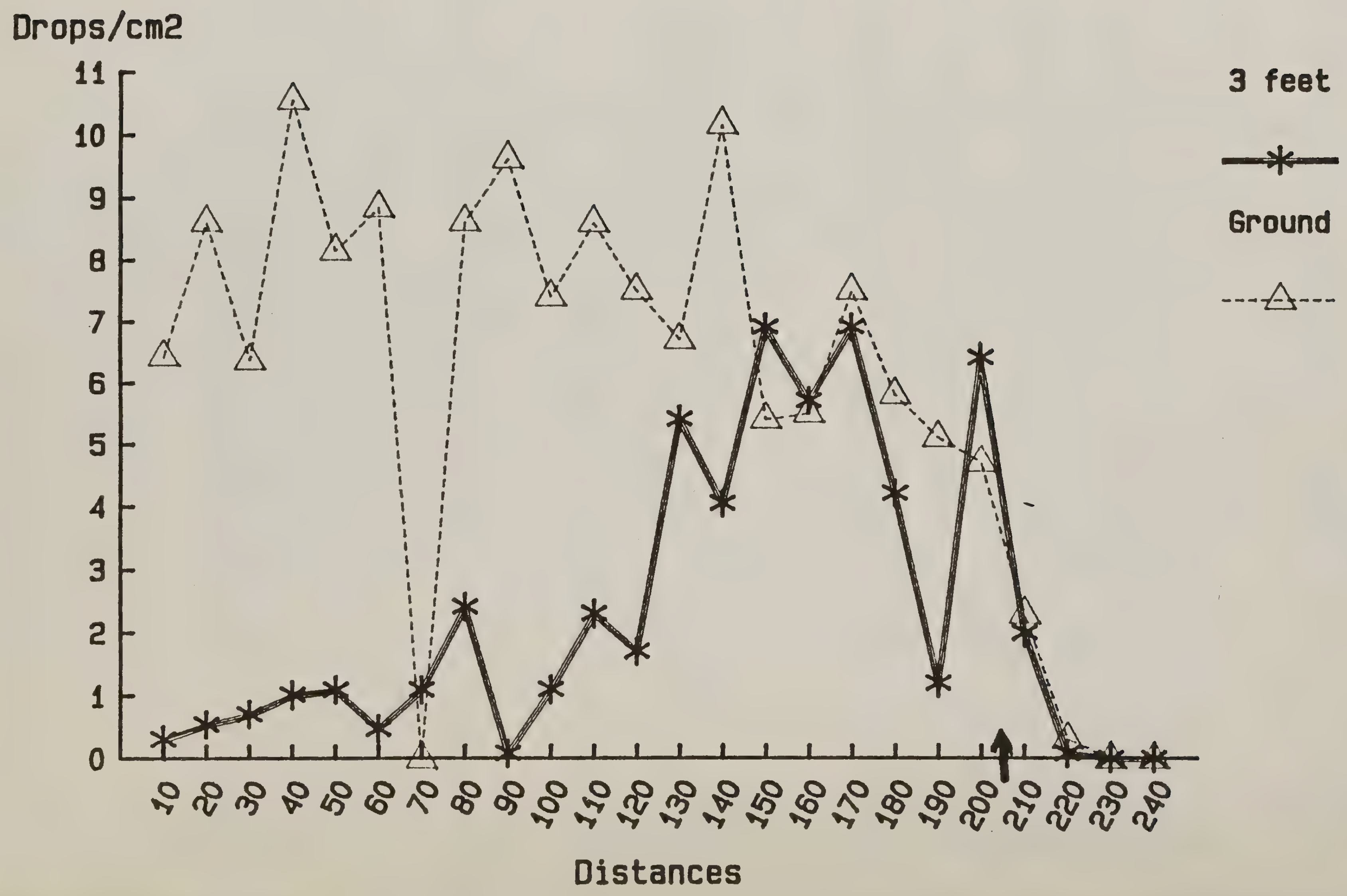
With techniques such as this, one may not acquire an accurate account of spray deposit. One is not sure if the collection device is the best nor if it should be placed on the ground or at a distance above the ground. Should the device be in a vertical or horizontal position? What is the actual droplet size and how many impacted per cm^2?

Studies were conducted during February, 1985, at the APHIS aircraft operations center at Moore Air Base, Mission, Texas. Six different collection devices were tested to determine the most effective one for trapping small droplets released by air. Devices were placed at ground level and 3 feet above ground level. Artificial leaves and Kromekote cards were placed in both a horizontal and vertical position. Ekblad samplers and small and large cylinders were in a vertical postion and petri dishes on a horizontal plane. All sampling devices were set out in an open field, arranged in 5 latin squares. Each device was replicated 8 times on each square.

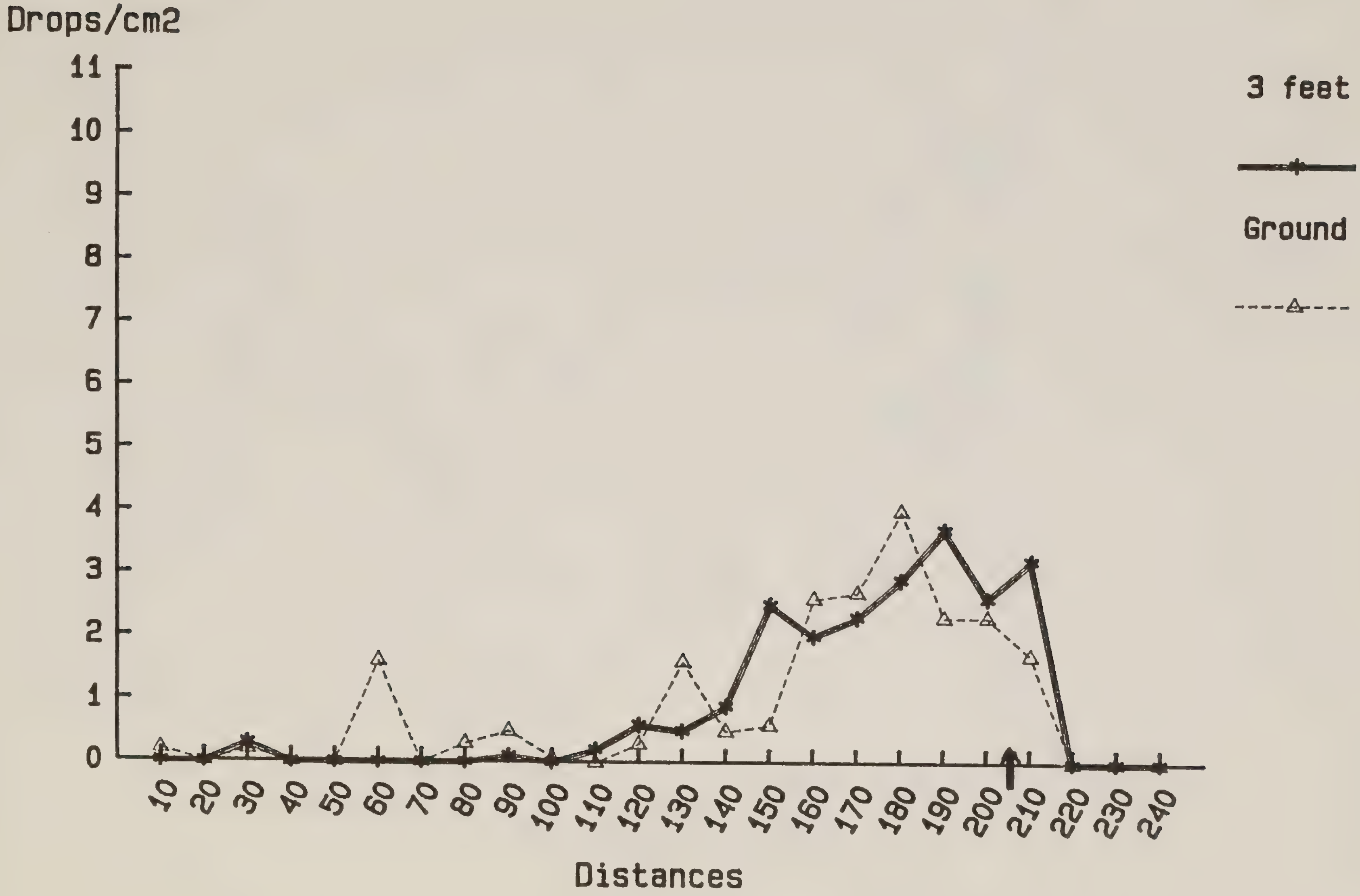
Thuricide 48LV and Dipel 8L were applied over the sampling area with a Cessna Ag-Truck aircraft, equipped with 8 AU-5000 Micronair nozzles. The materials were dispersed at 12 BIU/96 oz/acre, 50 feet above the target area. Sticker was not incorporated into the formulations. A total of 4 spray passes were made over the sampling area with each material. One hour after each pass, collection devices were retrieved and replaced with clean ones. Applications were attempted when wind conditions were minimal. However, we did have light to moderate wind during some of the applications.

All samples were sent to Davis, California, to be analyzed on a Quantimat 900. Work has been completed with the Quantimat 900 and results recorded on tape. Because of the vast amount of data, only a small portion is presently available. The following graphs represent results of one run with Thuricide 48LV.

MISSION, TEXAS
Horizontal Cards

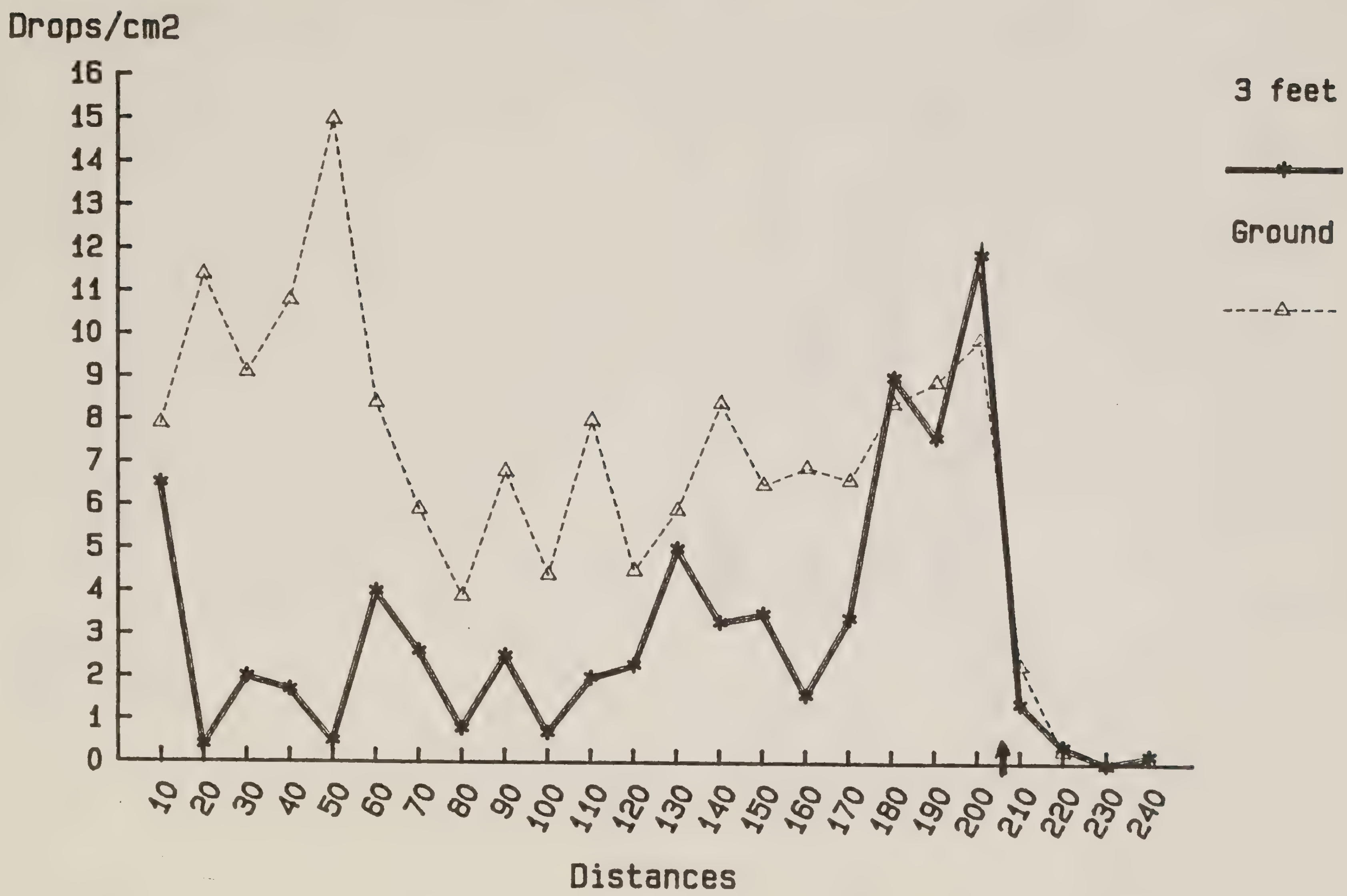


MISSION, TEXAS
Vertical Cards

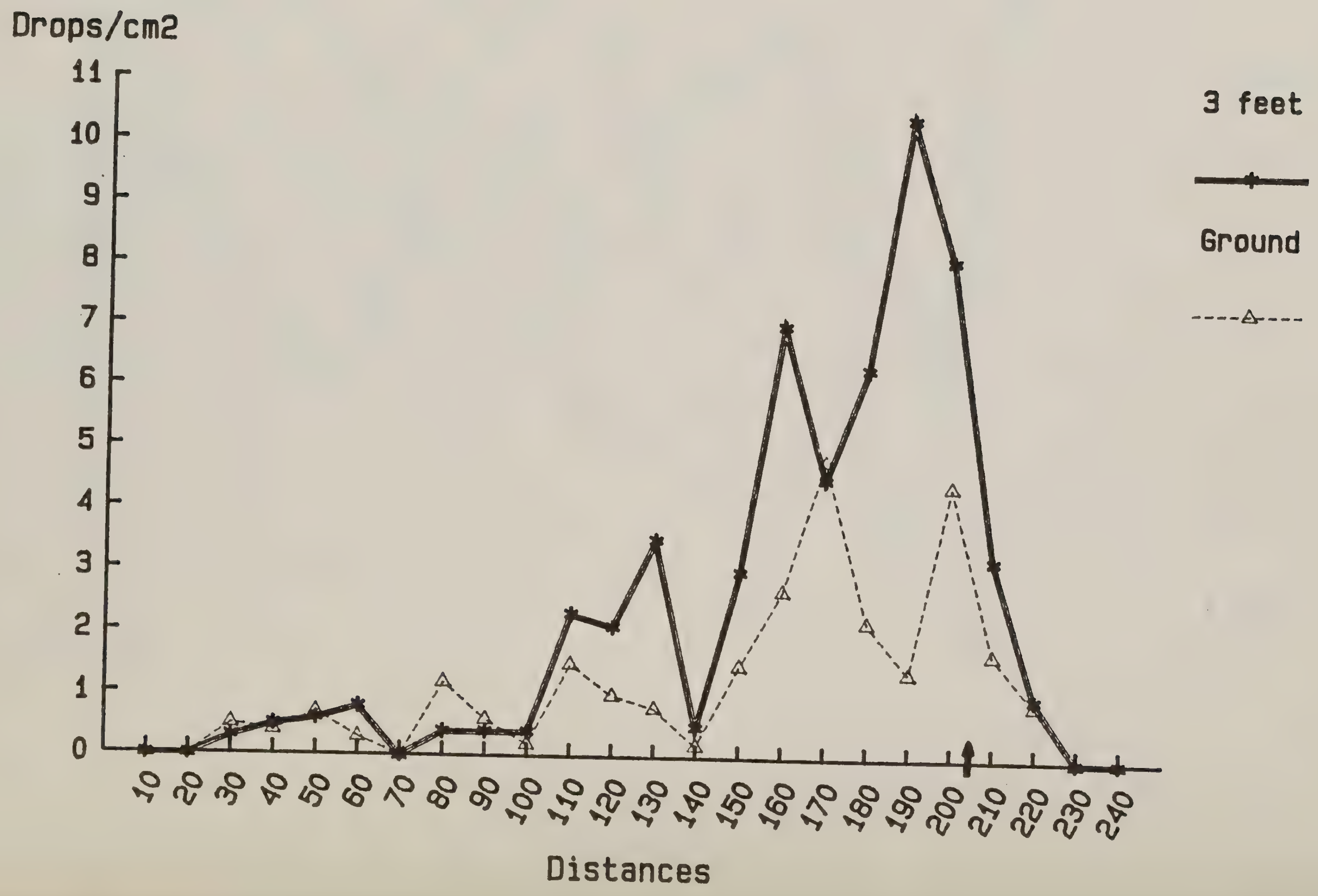


MISSION, TEXAS
Petri Dish with Kromekote Paper

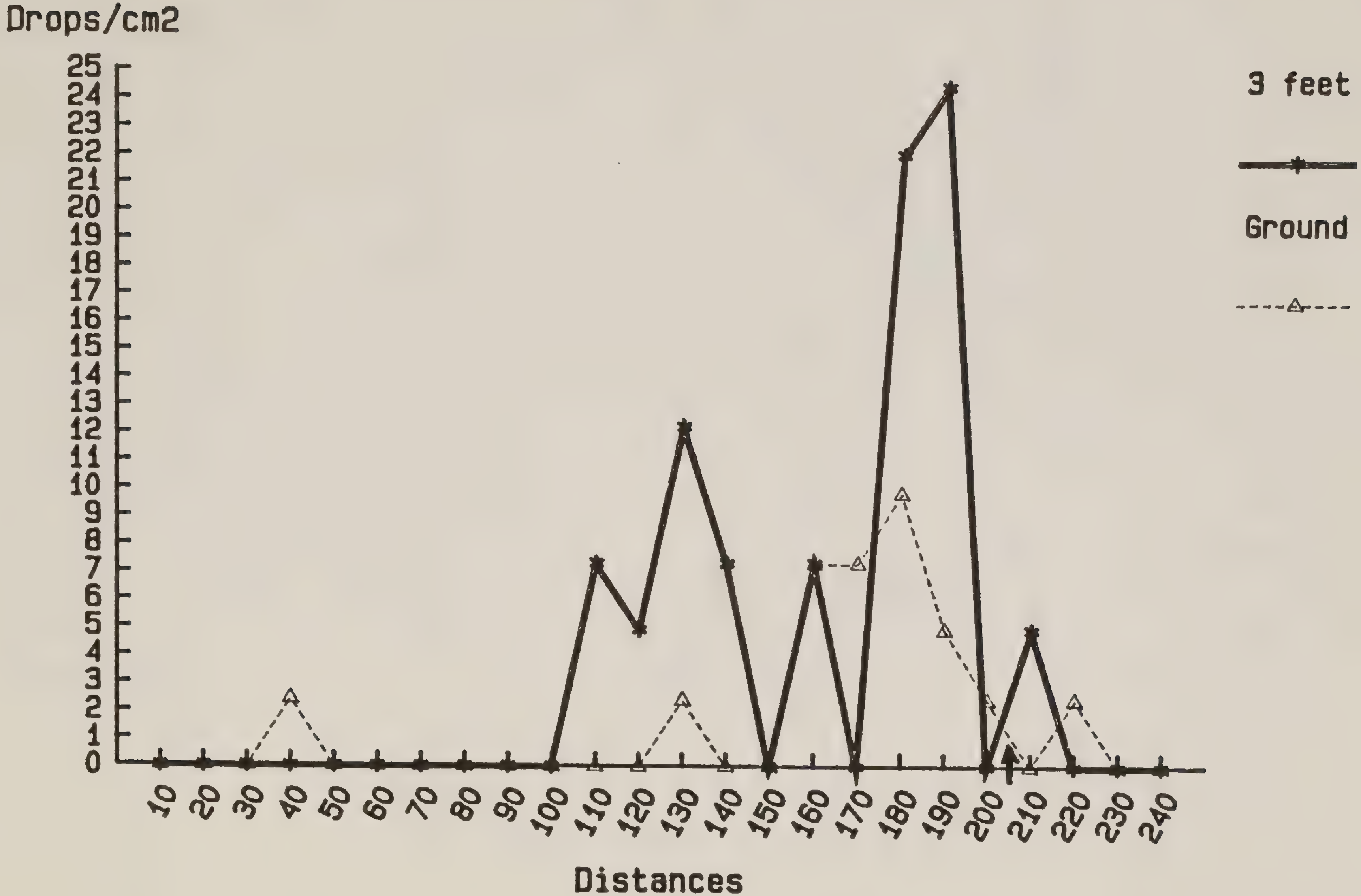
MISSION, TEXAS
Horizontal Leaves

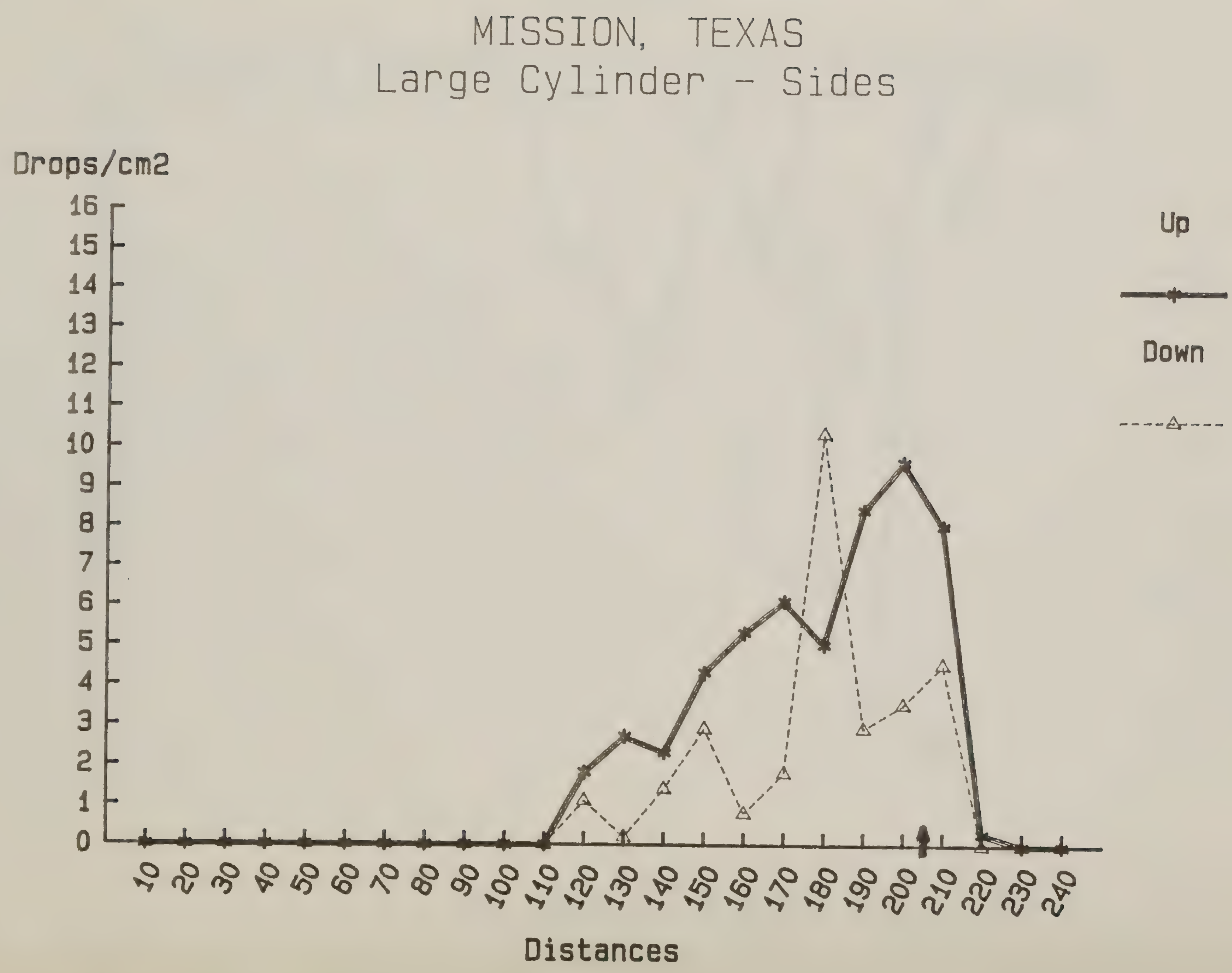


MISSION, TEXAS
Vertical Leaves

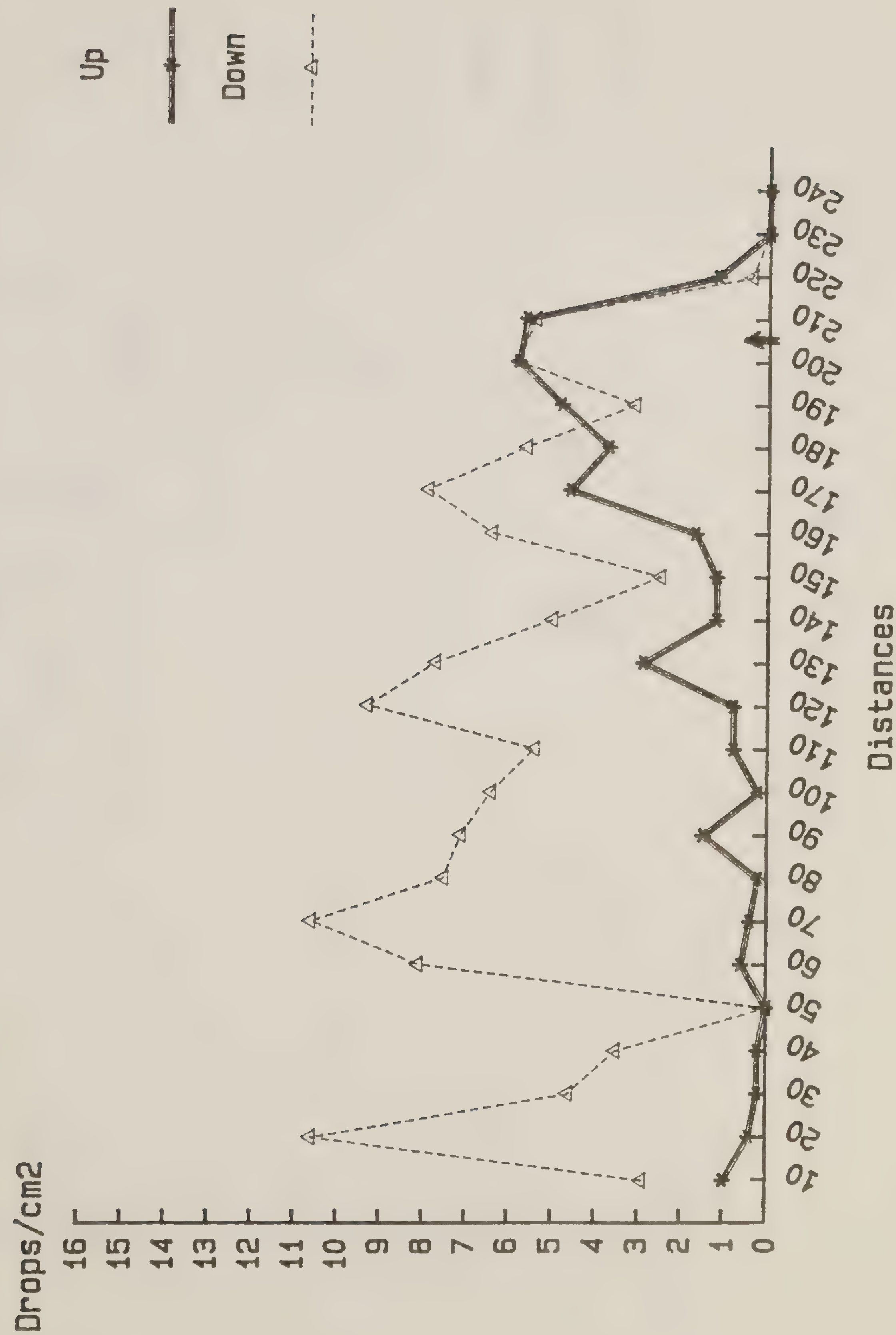


MISSION, TEXAS
Ekblad

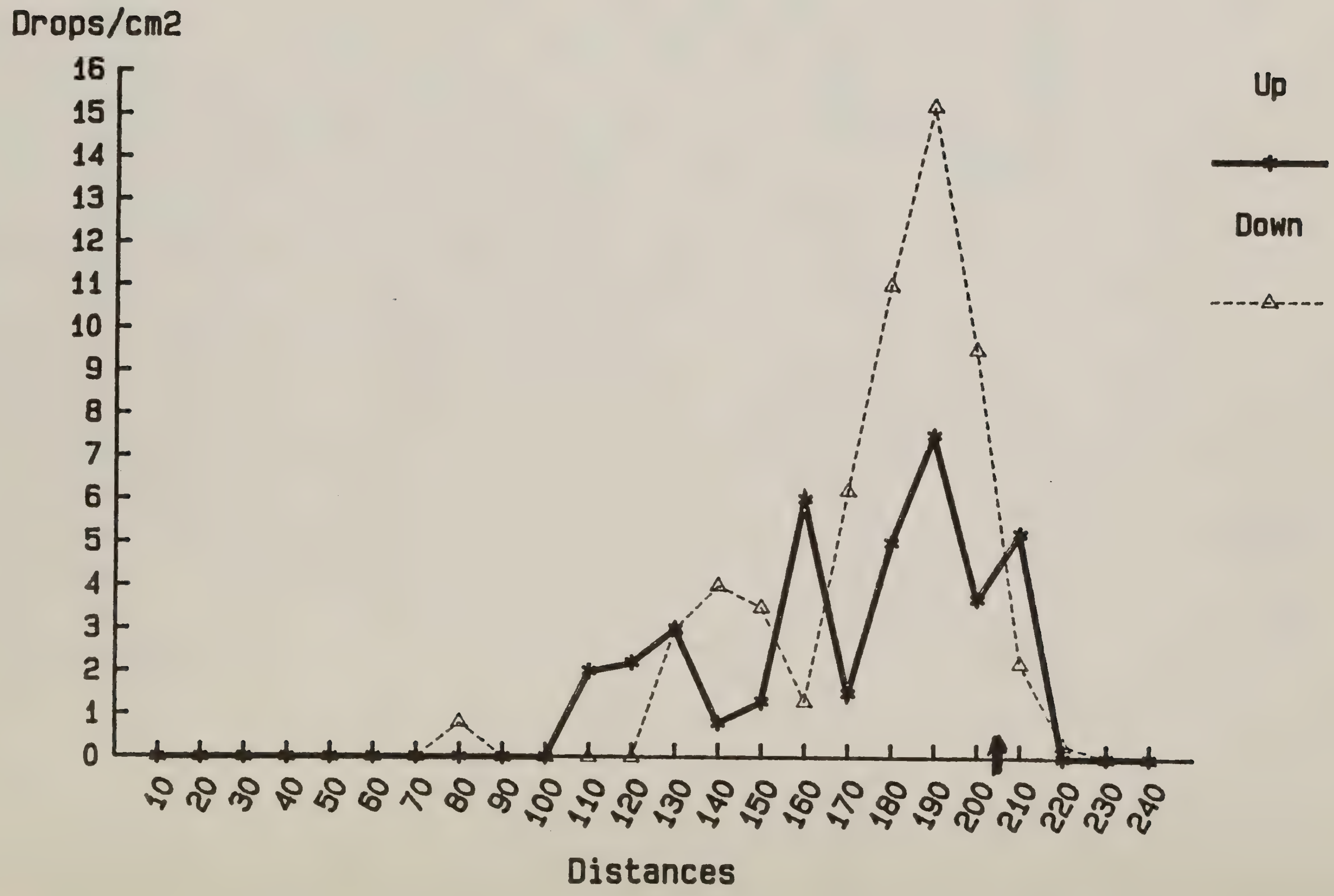



MISSION, TEXAS
Large Cylinder - Sides
Drops/cm2
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
Distances
Up
Down

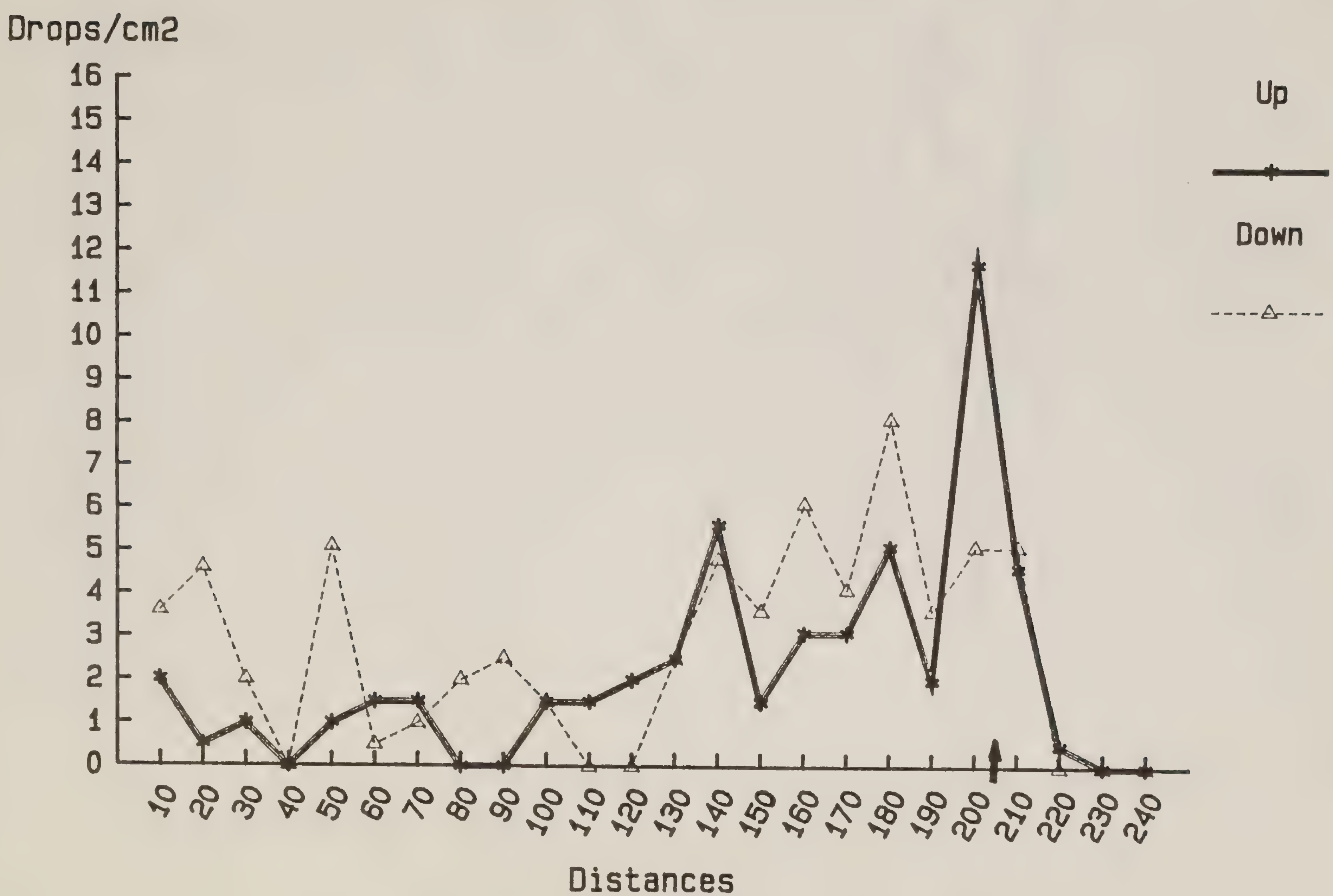
MISSION, TEXAS
Large Cylinder - Top



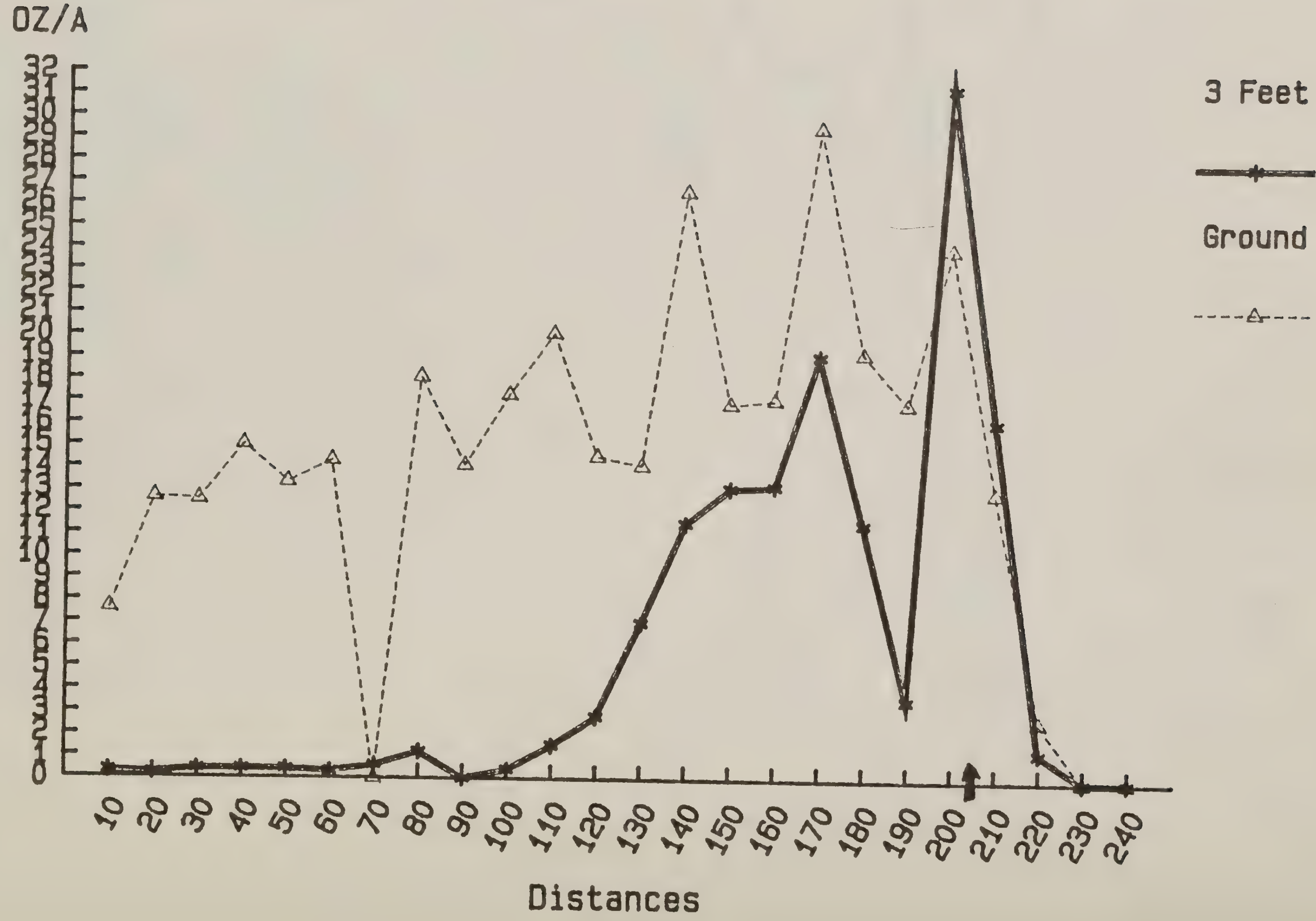
MISSION, TEXAS
Small Cylinder - Sides

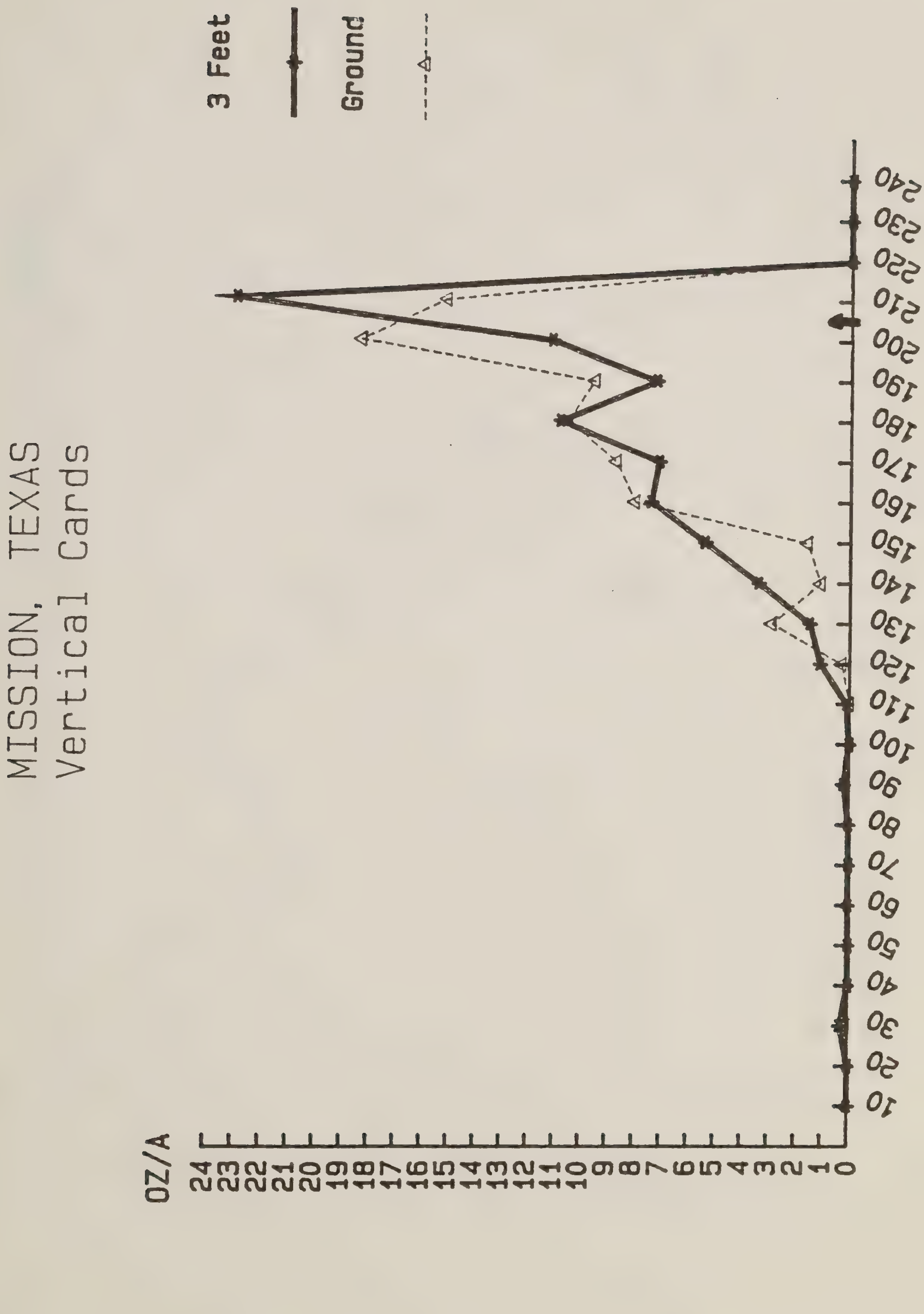


MISSION, TEXAS
Small Cylinder - Top



MISSION, TEXAS
Horizontal Cards




MISSION, TEXAS
Vertical Cards
3 Feet
Ground
OZ/A
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
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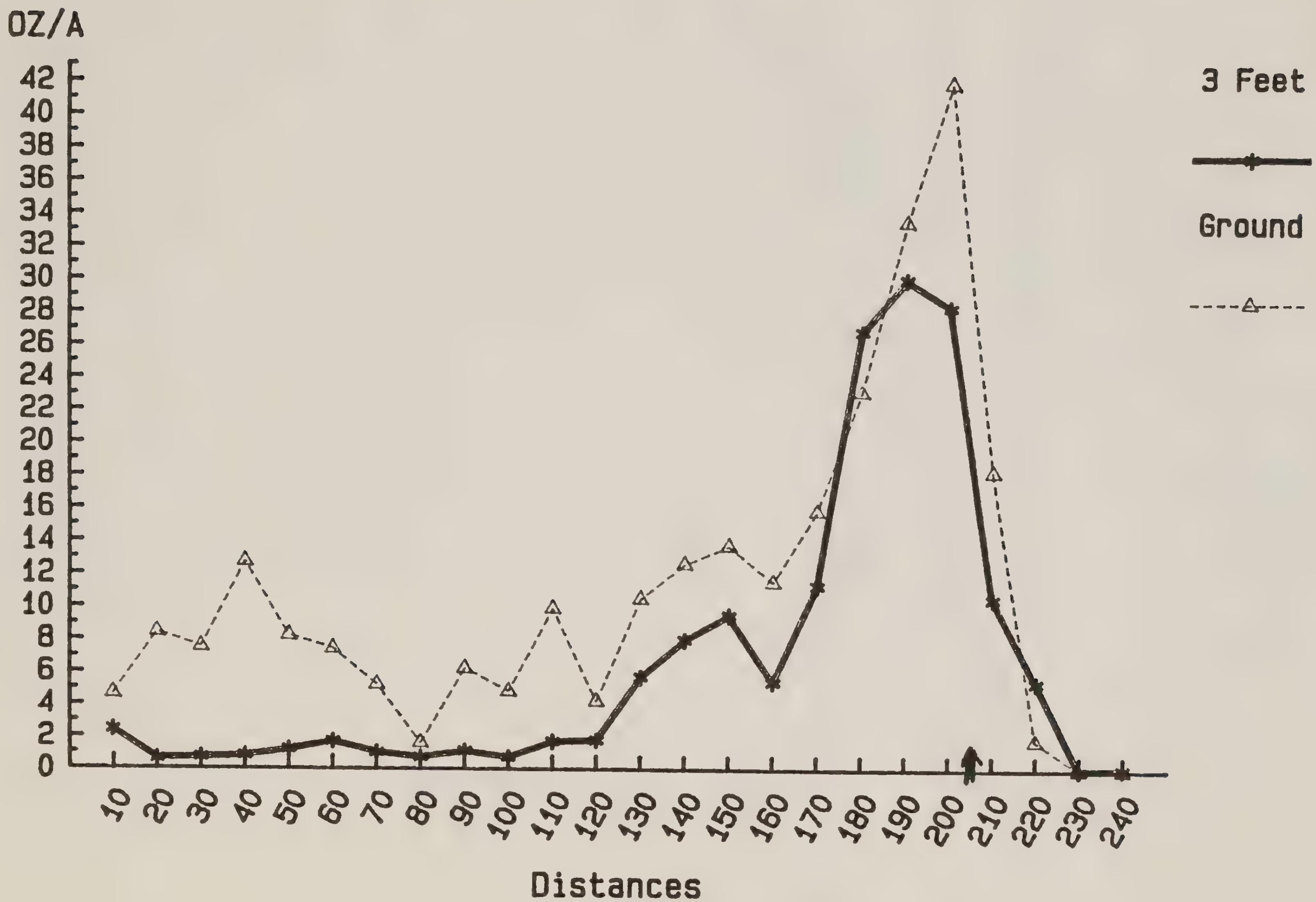
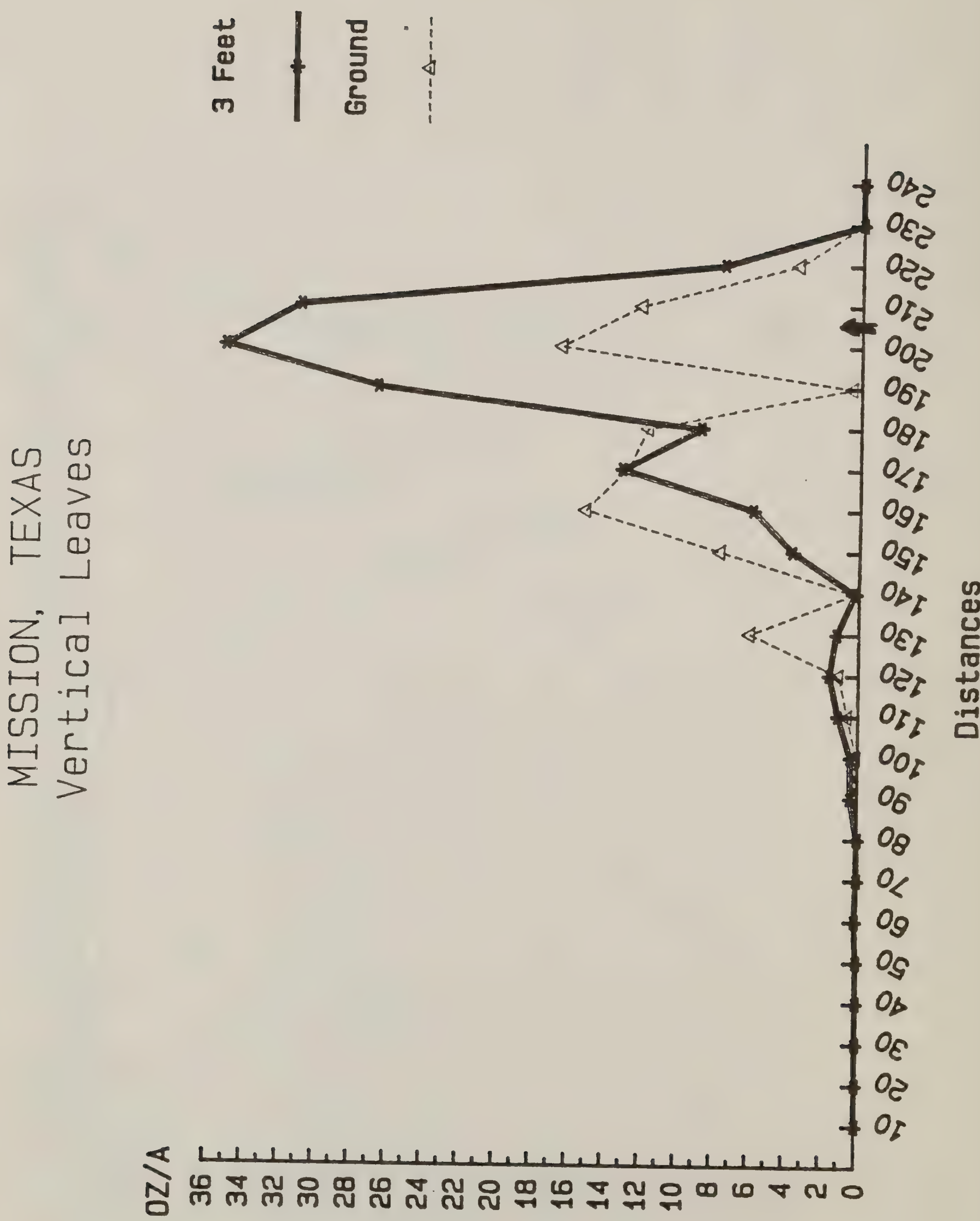
Distances

MISSION, TEXAS
Petri Dish with Kromekote Paper

MISSION, TEXAS
Horizontal Leaves




MISSION, TEXAS
Vertical Leaves
OZ/A
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
100
110
120
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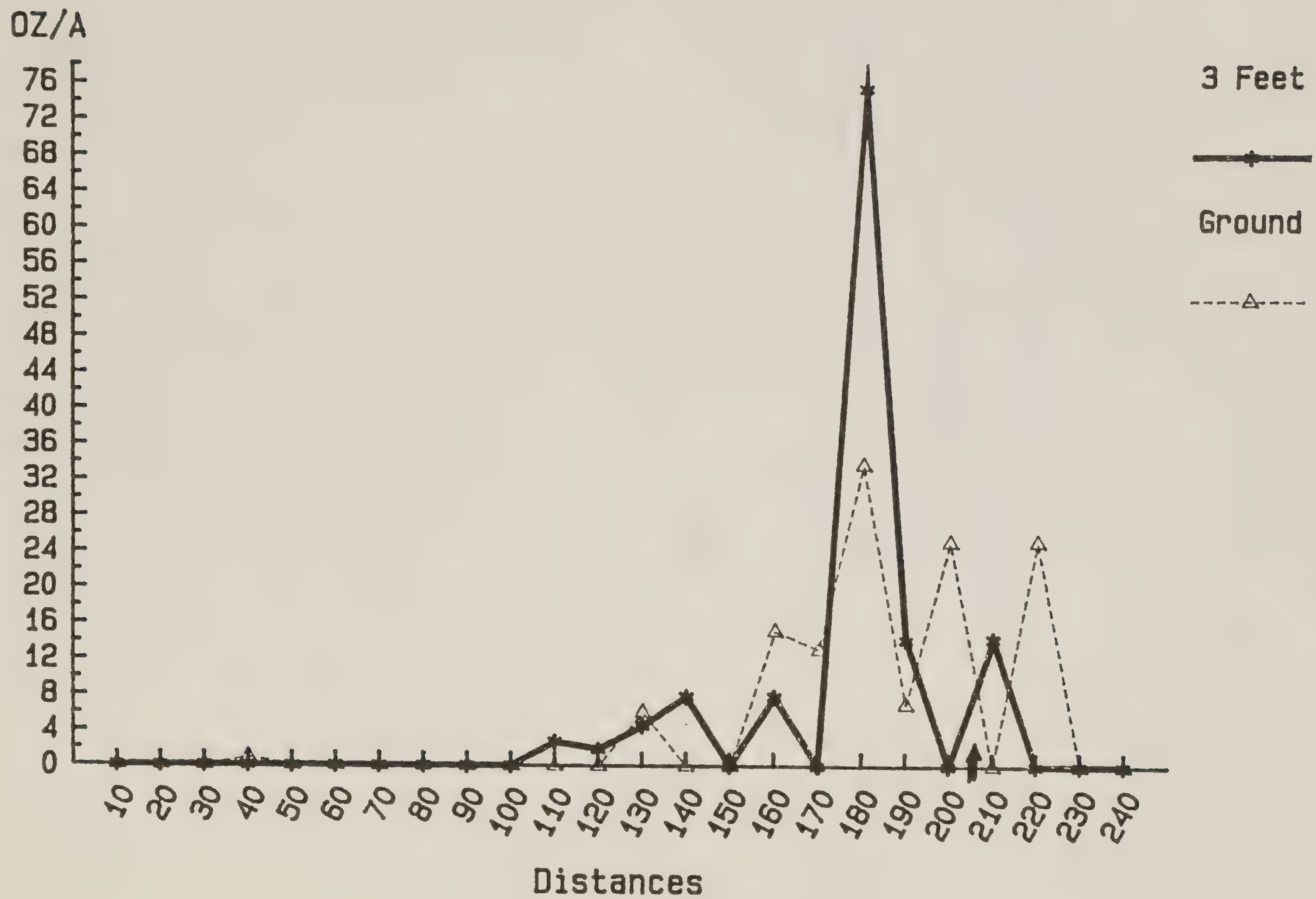
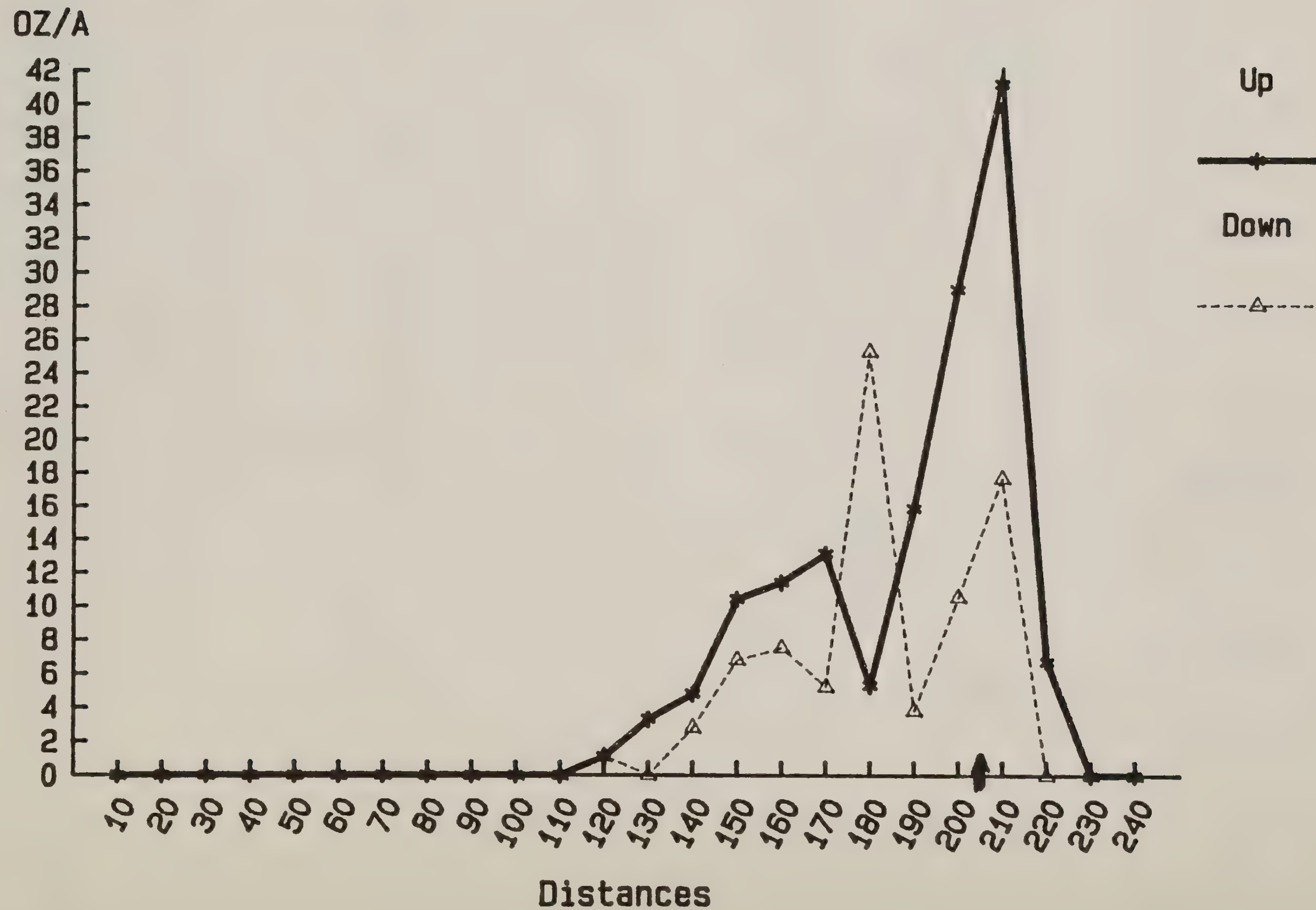
Distances
3 Feet
Ground

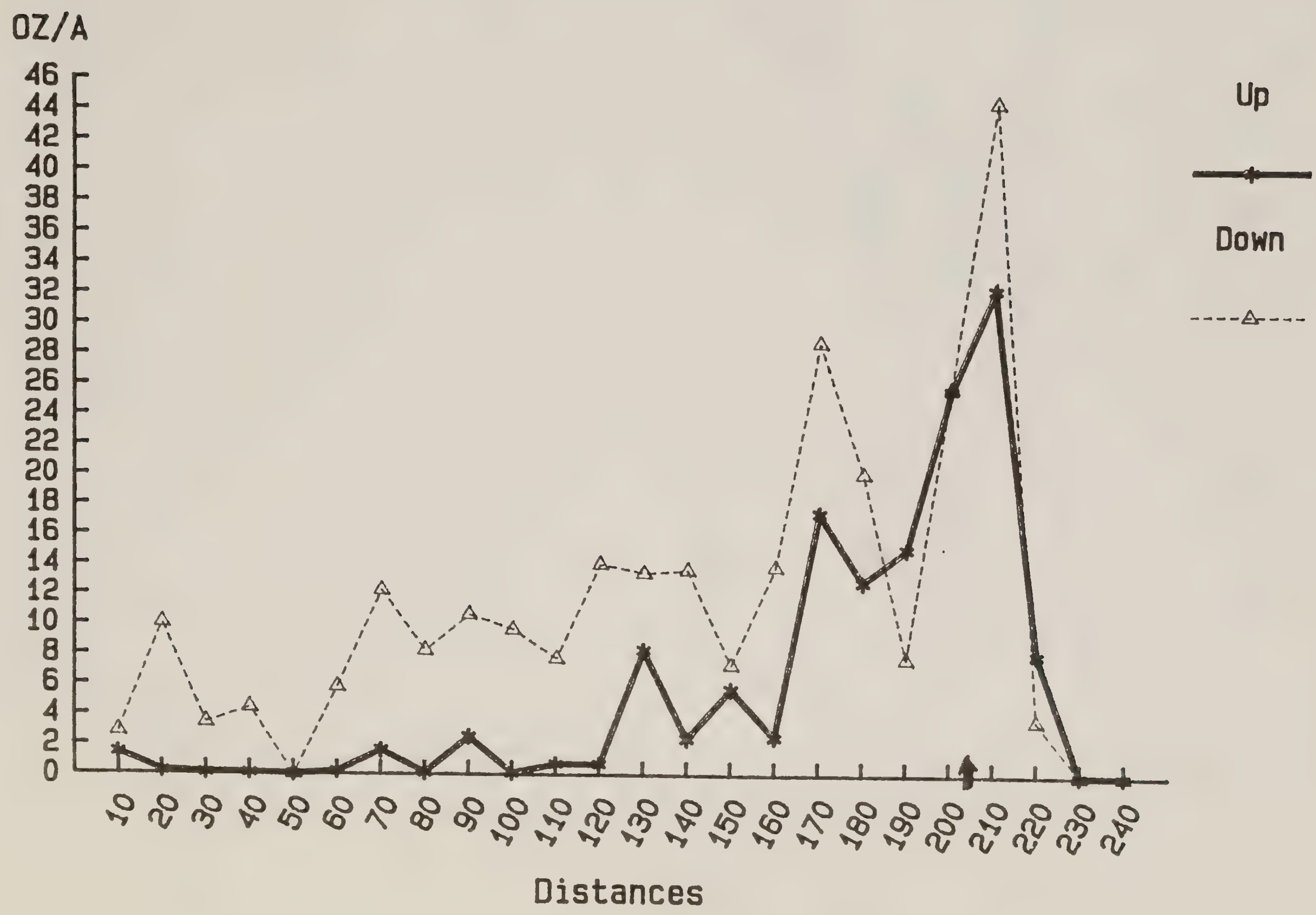
MISSION, TEXAS
Ekblad



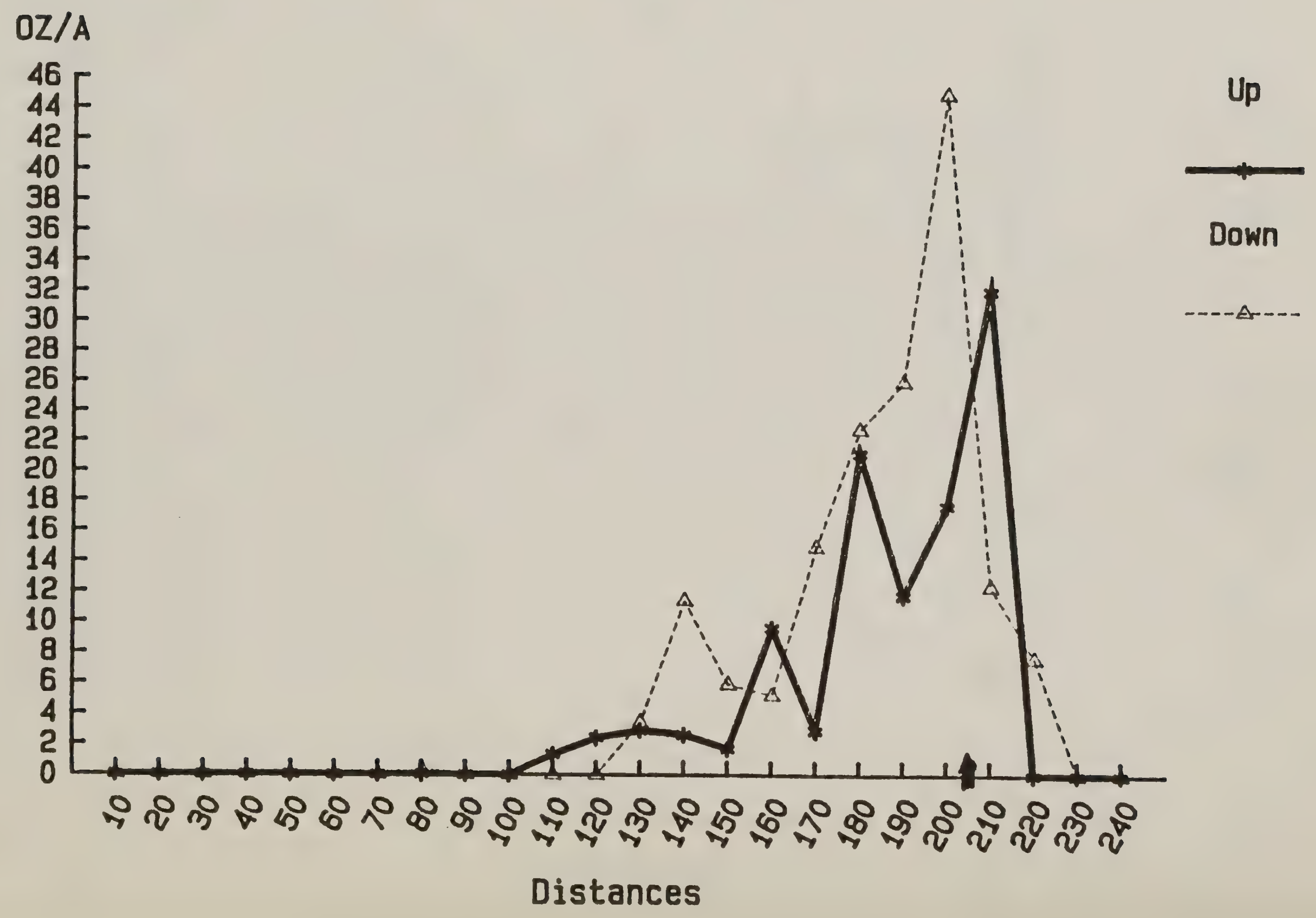
MISSION, TEXAS
Large Cylinder - Sides



MISSION, TEXAS
Large Cylinder - Top

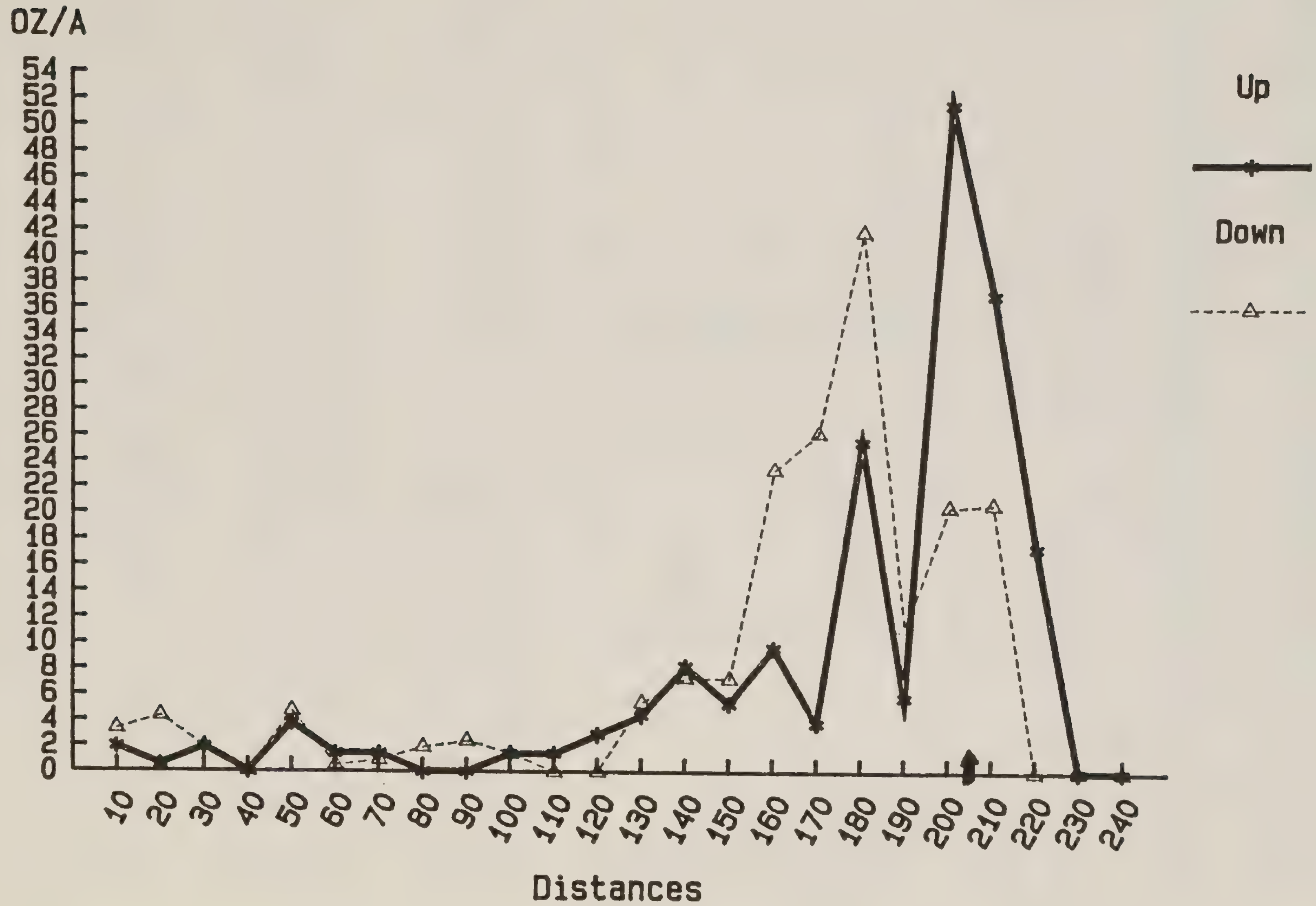


MISSION, TEXAS
Small Cylinder - Sides



MISSION, TEXAS

Small Cylinder - Top



Our 1985 field studies with Bt included a droplet size study. We wanted to produce droplet sizes of 100, 300 and 600 microns (VMD) with flat fan nozzles. At Mission, in February, we tested a number of different flat fan nozzle tips at various orientations to the boom. Droplet sizes were recorded from white Kromekote cards with a hand held measuring magnifier (Bausch & Lomb cat. # 81-34-35).

Table 1. Approximate droplet VMD produced with flat fan nozzles on a Cessna Ag-Truck flying at 120 MPH, 50 feet over the target and dispersing Dipel 8L at 12 BIU/96 oz/acre.

Nozzle size	PSI	Nozzle orientation to boom	Approximate VMD
8003	36	45° (Forward)	75-100
8003	36	90° (Straight Down)	75-100
8004	36	45°	100-150
8004	36	90°	150
8006	36	45°	150
8006	36	90°	200-250
8008	36	135° (45° aft)	250
8008	20	135°	300
8010	20	90°	400
8010	20	135°	400
8015	20	90°	600

Aircraft speed is the major factor in influencing droplet size. The orientation of the nozzle tip has only a minor influence on droplet sizes produced. The nozzles selected to produce 100 micron (VMD) droplets were 8003 at 45° forward, 300 micron (VMD) were 8008 tips at 45° aft and 600 micron (VMD) were 8015 tips at 45° aft.

In any spray program, off target drift has to be addressed. It is certainly advisable to monitor and eliminate the movement of spray deposit to sensitive areas outside of the target area. Improved sampling devices and techniques need to be developed for this purpose. Our goal should be to establish models that will predict drift trends under a varying number of meteorological conditions.

During September, 1985, a drift study was conducted at Mission, Texas. Cooperating in the study were USDA Forest Service, Pennsylvania State University and APHIS. A field test plot was set up with samplers utilized at various distances, out to 4,000 feet downwind, from the point of release from a Cessna Ag-Truck aircraft. Six sampling devices were utilized; kromekote cards, glass slides, Ekblad sampler, cotton string, small cylinders (straws) and active air samplers.

The aircraft applied at night (12:30 AM - 9:00 AM), with 8004 flat fan spray nozzles, Dipel 8L and Thuricide 48LV at 12 BIU/96 oz./acre. Nozzles were pointing 45^{o} forward to represent a standard operational application of Bt. No sticker was used, however, brilliant sulphaflavine dye was added to each tank mix to enable a more accurate evaluation of deposit.

All sampling devices were placed approximately 3 feet above the ground. Sample devices were collected one hour after spraying and replaced with clean ones. Single and 5 swath passes were made by the aircraft at the point of spray release.

All sampling devices were packaged and sent to Davis, California, to be analyzed on a Quantimat 900. At this time, results are not yet available.

Project Number: GM 5.1.3
Project Title: Viability of Gypsy Moth Eggs in Bark Mulch
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leader: E. C. Paszek and J. G. R. Tardif

Bark chips from logs infested with egg masses present a risk of spread out of quarantine areas. When these chips are composted by lumber mills, heat is generated in the bark mulch piles and, in the parts of the pile where heat is maintained for a sufficient period of time, the egg masses are destroyed. Egg masses were heat treated at temperatures of 100, 110, 120, 130 and 140°F for 30, 60, 90 and 120 minutes, by dry and moist heat methods. The temperatures of 120°F or greater, for 60 minutes, were consistently lethal (Paszek, Quarterly Report, July-Sept. 1969). Oven tests of egg masses attached to various substrates (aluminum, steel, wood, spun glass and celotex) indicated total mortality results from 100°F for 3-1/2 hours, 120°F for 1-1/2 hours or 130°F for 15 minutes (Merrian and Stevens, Progress Report, 1/20/71).

In this study, diapause completed laboratory reared and field collected egg masses, both intact and fragmented, were placed with 10 oz. of moist bark mulch in 7 x 8.5" mylar bags that were heat sealed. Two holes, rhombus shaped (adjacent sides 1/4" long), were punched in each bag to allow for the escape of moisture. The bags were held at room temperature and in environmental cabinets at ambient, and 100, 120, and 140°F for 1, 2, 3, 4, 5, 7 or 14 days. Lab reared and field collected controls were placed in petri dishes. They were stored in the screen house from 4/19 to 5/6/85, and brought indoors to room temperature on 5/6/85. The results of this experiment are summarized in Table 1.

Table 1. Post diapause egg masses held in mylar bags containing bark mulch and heat treated in environmental cabinets.

		Percentage of Masses Hatched in Mylar Bags				Percentage of Controls Hatched in Petri Dishes		
No. Masses	Temp.	Tmt. Days	L.R. Intact	F.C Intact	L.R. Frag.	No. Masses	L.R. Intact	F.C. Intact
14	Amb.1/	0	7	0	---	22	100	100
5	100°F	1	100	100	100			
5		2	60	80	30			
5		3	20	20	20			
5		4	0	40	0			
5		5	0	0	0			
5		7	0	0	0			
5		14	0	0	0			
25	120°F	1-5	0	0	0			
5		7	0	0	0			
5		14	0	0	0			
25	140°F	1-5	0	0	0			
5		7	0	0	0			
5		14	0	0	0			

1/ Stored in screenhouse, brought indoors on 5/6/85, date treatment commenced.

100°F for 4 days was the minimum lethal temperature for killing lab reared, intact and fragmented egg masses. For the field collected egg masses, the minimum lethal temperature was 100°F for 5 days. The controls, both lab reared and field collected, had 100% hatch in the petri dishes. Field collected masses held at ambient temperature in mylar bags with bark mulch, had 100% mortality, while mylar bags with lab reared masses had 7% hatch. One egg mass out of 14 had partial hatch. It is clear that the minimum lethal temperature of 100°F for 5 days would kill post diapause gypsy moth egg masses in a composting bark mulch pile.. What prevented the masses from hatching in the mylar bags packed with moist bark mulch and held at ambient temperature was not clear, but ovicidal effects of bark mulch was indicated. The 100°F temperature for 1 day speeded up the incubation resulting in all the masses hatching. As the 100°F temperature was extended from 2 to 4 days, the percentage of masses that hatched was reduced, due to the ovicidal effects of the bark mulch.

A follow-up experiment on similar post diapause lab reared egg masses that were used for controls (Table 1) was set up as follows:

1. Packed in mylar bags with 10 oz. of moist bark mulch and heat sealed.
2. Placed in mylar bags without bark mulch and heat sealed.
3. Placed in covered petri dishes.

These egg masses were brought in from the screen house on 5/10/85 and the hatching data are summarized in Table 2.

Table 2. Post diapause laboratory reared egg masses held at ambient temperature in mylar bags with and without bark mulch.

Percentage of 5 Masses Hatched in Mylar Bags		Percent Hatch of 5 Controls
With Bark Mulch	Without Bark Mulch	In Petri Dishes
0	100	80

The egg masses packed in mylar bags with bark mulch did not hatch, while the egg masses in mylar bags without bark mulch had 100% hatch. Four egg masses in the petri dishes hatched 100% and one had approximately 25% hatch. Bark mulch evolves ovicidal agents that kill the embryos in the eggs. The egg masses in this experiment were placed inside the bags on 5/10/85 and the egg masses began to hatch in the bags without bark mulch and in the petri dishes on 5/14. Egg masses buried in bark mulch piles succomb to heat and ovicidal agents (caused by the partial decomposition of the bark) that are generated in the pile.

A new bark mulch pile 30' wide, 15' high was made on 3/25/85 from fresh white pine logs that were debarked by a ring debarker at the Lorden Lumber Co., Inc, in Milford, New Hampshire. In this mulch pile 8 equidistant points were designated and temperature measurements were taken at 8 intervals, 3/29 to 5/7/85, with a heat measuring probe or with set thermocouples at 3, 6, 9 and 12' depths and at heights of 1.5, 3, 4.5 and 6'. Post diapause egg masses were dehaired and the eggs were placed inside perforated wooden capsules and inserted in the pile 4/4/85 at a 4.5' height between the four cardinal points at 3' and 6' depths. Temperature recordings were made at 8 intervals from 4/4 to 5/7/85. Control capsules with eggs were placed at the edge of the west side of the pile and protected from sunlight.

Additional capsules with post diapause eggs were inserted into the pile on 4/29 and removed on 5/3 and 5/7/85. The capsules containing post diapause eggs that were inserted into the pile at the 3' depth, between four cardinal points on 4/4/85, hatched from the NE, SE and SW locations. The eggs in the NW location, which reached a temperature of 33.8°C did not hatch. In the 6' depth none of the eggs hatched. The two controls from the west side of the pile hatched (Table 3). The post diapause eggs that were inserted into the pile for 4 days (4/29-5/3/85) and 8 days (4/29-5/7/85) indicate that 4 days is not a long enough period of time to kill eggs at the 6' depth; eggs at this depth were killed in 8 days (Table 4). Eggs at the 3' depth generally hatch unless a lethal temperature is attained or they are exposed to other ovicidal agents from the bark.

Special thanks to Mr. Kenneth Lorden, President of the Lorden Lumber Company, Inc. in Milford, New Hampshire for setting up a bark mulch pile and allowing us to conduct experiments on his property.

Table 3. Internal temperature of bark mulch pile and hatch of egg masses buried near the point where tempertaure readings were made.

Sample Location	Depth	Dates Temperature Taken 4/4	4/8	4/12	4/17	4/24	Hatch
NE	3'	11.4	26.1	19	22.7	27.2	Yes
	6'	34.2	52.7	55.2	51.0	41.7	No
SE	3'	5.2	15.0	14.0	25.2	14.8	Yes
	6'	18.2	34.1	37.3	31.7	28.0	No (moldy)
SW	3'	17.7	32.0	28.2	31.1	30.7	Yes
	6' 1/						
NW	3'	11.1	25.8	20.5	42.8	33.8	No
	6' 1/						
W. Edge 2/	(Control #1)						Yes
	(Control #2)						Yes

1/ Capsule lost while being pulled from pile.
2/ Control capsule kept out of sunlight.

Table 4. Hatch of cleaned, post diapause eggs in capsules held in bark mulch pile for 4 and 8 day intervals.

Sample Location	Depth	^{o}C on Date of Insertion	No. Days in Pile	Hatch
N	3'	38.3	4	Yes-partial
E	6'	42.8	4	Yes-partial
Top Center	3'	45^{o}C	4	No
Top Hot Spot	3'	Steaming 1/	4	No-boiled
W - Control	edge 2/	Ambient	4	Yes
N	3'	28.8	8	Yes-partial
	4'		8	No
	6'	32.5	8	No
Top Center	3'	45^{o}C	8	No
Top Hot Spot	3'	Steaming 1/	8	No-boiled, moldy
W Control	Edge 2/	Ambient	8	Yes

1/ Temperature in steaming hot spot (vent hole) fluctuates 53^{o}C - 80^{o}C.
2/ Controls were placed in western edge of pile, out of sunlight.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Gypsy Moths
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: V. C. Mastro

Studies to determine the effects of treating both sexes of gypsy moth with low doses of radiation have been initiated. To date, no results are available for reporting.

Project Number: GM 1.2.4
Project Title: Mass Trapping
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: C. P. Schwalbe, V. C. Mastro

No additional work has been performed on this project as this method for eradicating isolated infestations is gaining acceptance and credibility in states not generally infested. Indeed, multiple applications of BT, followed by mass trapping is the strategy of first choice in some states. The trap density necessary for effectively preventing female mating is a function of population density and since target population density (and sex ratio) cannot be accurately predicted, some guesswork is needed to devise a trapping plan. Results of studies and operational experience suggest that 25 traps/ha will prevent most mating in populations of 25 pairs/ha/day. Population densities higher than that are rarely found in isolated infestations and, therefore, 25 traps/ha is the recommended density for most situations. As our experience with this technology grows, it is probable that situations will be defined under which lower trap densities will be effective.

The important features that influence the effectiveness of mass trapping in preventing mating are:

1. The technique is highly population density-dependent, but it appears that most mating is prevented with a trap:mating pair ratio of 1:1 (at populations of 2.5-25 pairs/ha).
2. Males are captured in traps earlier in the day than females are mated, giving the traps a decided "edge" over females.
3. An important consequence of mass trapping is the reduced incidence of multiple-mating by male moths. Over 50% of the mating in control plots was due to males that mated up to 4 times. Multiple mating in trapped plots was rare.

A manuscript is in preparation which will summarize the work connected with this project. For review, see GM 1.2.4 in 1981, 1982 and 1983 and GM 2.2.5 in 1982, 1983 and 1984 reports.

Project Number: GM 2.2.4
Project Title: Induced Inherited Sterility Trial - Horry Co., South Carolina
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: V. C. Mastro and T. M. ODell

This project objective was to evaluate the feasibility of eradicating an isolated population of gypsy moths using releases of substerile males (ie. treated with 10 Krads as 8-11 day old pupae), thereby inducing sterility into the native F_1 population. Crosses of released males treated with 10 Krads of radiation and fertile wild females result in sterile progeny the year following release. Males were released in 1982, and the site was monitored in 1982 and 1983 for overflooding ratios and the impact of sterile F_1 progeny. In 1984 and 1985, to confirm eradication, the area was trapped (32 traps/square mile). No males were trapped in either year. Therefore, we conclude that the population was eradicated and the objective achieved.

Project Number: GM 2.2.5
Project Title: Mass Trapping Pilot Study - Monona, Wisconsin
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: C. P. Schwalbe, V. C. Mastro

In 1981, a small, isolated gypsy moth infestation was located in Monona, Wisconsin. Because the infestation was adjacent to a lake (Monona) and in a residential area, it was resolved to attempt eradication through the use of mass trapping. Details of the history (with maps) are in 1982 and 1983 Progress Reports. It should be noted that the reduction in male density observed from 1981-82 was probably due to extremely cold 81-82 winter temperatures. The following is a summarization of data collected since the project began:

Table 1. Results of male moth trapping and larval and pupal sampling (under systematically placed burlap bands) in Monona, Wisconsin.

	1981	1982	1983	1984	1985
Number male moths captured	236	113	40	7 5/	0 5/
Estimated male population	2360 1/	151 2/	53 2/	8 6/	0
Number larvae and pupae		31	34	0	0
Estimated ha infested		13	8	1	0
Estimated number of pairs (season-long)/ha		11.6 3/	6.7 3/	8.0 3/	0
Flight period (days)		21	21	21	-
Estimated pairs/ha/day		0.6	0.3	0.38	-
Estimated number females mated		13.3 4/	4.7 4/	0.48 7/	-

1/ Assuming 10% capture rate with 32 traps/mi^2
2/ Assuming 75% capture rate with 7.5 traps/ha
3/ Assuming 1:1 male:female sex ratio
4/ Number females mated/season = (no. pairs/ha/day)(.088)(flight period)(ha infested) where .088 = mating success of 2.5 females/ha in area containing 7.5 traps/ha
5/ 25 traps/ha deployed in 1984 and 1985
6/ Assuming 86% capture rate with 25 traps/ha
7/ Number females mated/season = (no. pairs/ha/day)(.06)(flight period)(ha infested) where .06 = mating success of 2.5 females/ha in area with 25 traps/ha

Since calculations indicate less than one female was mated in 1984, eradication of the population was predicted. 1985 trapping resulted in no male moths caught, strongly suggesting that no reproduction took place in 1984. This pilot project is the first systematic attempt to eradicate an isolated infestation solely through the use of mass trapping. Numerous other examples (from other States) of the disappearance of an infestation following mass trapping are available, but they were not evaluated with burlap collection of immatures and other actions taken complicate drawing cause - effect relationships.

Project Number: GM 3.2.2
Project Title: Partially Sterile Male (F_1) Pilot Study Kent County, Maryland
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, K. Tatman, T. M. ODell and R. E. Webb

The objective of this project was to evaluate the impact of a release of sub-sterile males (irradiated as pupae, 8-11 days old, with 10 Krads) on a native gypsy moth population. In 1983, approximately 9-10,000 male pupae were released daily in a 96 acre woodlot. To monitor the impact of the release, pre- and post-season egg mass counts were taken, larval and pupal surveys were conducted and male adult flight and mating ratios were monitored using traps and placed monitor females. Sample plugs were also taken from feral egg masses at the end of the field season to determine the actual sterile:fertile (S:F) mating ratio. To monitor the carry-over effect in the F_1 generation, in 1984, larval and pupal samples were collected from the field and reared in the laboratory to the adult stage then mated to normal laboratory stock for determination of type (i.e. F_1 sterile or wild fertile). Monitor females and pheromone baited traps were also placed to monitor mating ratios and male flight.

Egg masses oviposited by monitor females placed in 1983 were evaluated to determine male mating types (i.e. released 10 Krad males or wild males). Preliminary results of this evaluation were reported in the last Annual Report. A final analysis of the 1983 monitor female egg masses determined that in 17 of the matings, the male parent was wild and in 345 of the matings the male parent was a released 10 Krad treated male (Table 1). Determination of mating type, however, when these two types of male parents are possible is not totally accurate. Egg mass evaluation for mating type is based on the proportion of eggs in a mass which are embryonated and the proportion of embryonated eggs which hatch. Because of the variability of these characteristics in both types of matings (10 Krad irradiated male X untreated female and untreated male X untreated female), there is considerable overlap in the degree of embryonation and hatch for these two mating types.

To determine what the error rate was in the evaluation of 1983 monitor female egg masses, F_1 progeny from some of the 1983 monitor female egg masses were reared to the adult stage and back mated to normal laboratory reared insects. In all, progeny from 120 monitor female egg masses were available for rearing and mating. When possible, three progeny from each egg mass were outcrossed and the resulting F_2 egg masses evaluated. When an F_1 male progeny was involved in the outcross, the evaluation was based on both the proportion of eggs which were embryonated and the proportion of embryonated eggs which hatched. When an F_1 female progeny was involved in the outcross, the evaluation was based only on the proportion of eggs which hatched.

Egg mass evaluations of 95 F_2 families were compared with the original evaluations of egg masses oviposited by monitor females (F_1 egg masses). In 53 of the cases where the F_1 egg mass was judged to be the result of a mating between a monitor female and a released 10 Krad male, the F_2 egg mass evaluation agreed. In 19 cases where the evaluation of the monitor female F_1 egg mass was determined to be the result of a 10 Krad male mating, the F_2 egg mass evaluation determined that the male involved was wild (and the original evaluation was in error). In 2 cases where the evaluation of the F_1 egg mass could not determine the male parent, evaluation of the F_2 egg masses determined that the male type was 10 Krad male and in 1 case where the initial evaluation judged that the monitor female was mated with a wild male, F_2 egg mass evluation determined the male type was irradiated (10 Krad male). In 20 cases, F_1 progeny produced egg masses which did not contain any eggs and in these cases a comparison was not possible.

From these data, the error rate in judging mating type for monitor females (untreated), when the two possible male parents are wild (fertile) or irradiated (10 Krads), can be calculated. Including only F_1 egg masses which were judged to be the progeny of an irradiated male, approximately 24% of the time the evaluation would be in error and 76% of the time it would be correct. If this error rate is applied to the initial evaluation of egg masses produced by monitor females, of the total 348 egg masses which were originally considered progeny of irradiated males, only 264 would be correct and 84 were really the progeny of wild, fertile males.

Table 1. Mating type determinations of egg masses deposited by feral females and laboratory females placed as monitors in 1983 in the Kent County treatment plot (plot 2).

Female Type	No. of Egg Masses of Each Mating Type: Feral Male	No. of Egg Masses of Each Mating Type: Released 10 Krad Male	Sterile: Fertile Ratio	No. of Egg Masses Parentage Unknown
Monitor Females	17	348	20.4:1 (2.6:1)1/	8
Feral Females	1	69	69:1 (2.9:1)1/	1

1/ Adjusted overflooding ratio based on fertility of F_1 progeny.

Adjusting the overflooding ratios to compensate for these errors in the original evaluation of egg masses, results in a calculated 2.6S:1F overflooding ratio for egg masses oviposited by monitor females and a 2.9S:1F overflooding ratio for feral females. Both of these estimates of mating ratios are considerably lower than the original evaluation of F_1 egg masses indicated. (Table 1).

Male trapping during the 1983 field season indicated the overflooding ratio for the entire field season was about 10.4S:1F. On several days during peak native flight, this ratio was much lower and on 2 days more feral males than released males were captured. The average overflooding ratio, during peak native flight (July 9-22) averaged only 3S:1F (Figure 1). An overflooding ratio of 2.5S:1F during the 1983 field season would only be expected to produce a 1S:1F ratio in the 1984 field season.

In the Kent County plots during the 1984 field season, trees were burlap banded within the 1983 release site (plot 2) and in one control site (plot 4). During the 1984 field season, pupae were collected from under bands and shipped to the Otis Methods Development Center Laboratory. Upon eclosion, these insects were back mated to normal laboratory insects and the resulting egg masses were evaluated for degree of embryonation and hatch. Monitor females were also placed during the 1984 field season. Egg masses oviposited by them were also evaluated for degree of hatch and embryonation.

Although results of egg mass evaluation from both field collected pupae and monitor females is not complete, preliminary indications are that very few sterile F_1s were present during the 1984 field season. Final results and analysis of this project will be presented in the next Annual Report.

Project Number: GM 3.2.5
Project Title: Sterile Male Techinque - Studies on the Feasibility of Releasing F_1 Sterile Gypsy Moths as Eggs.
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, J. D. Tang, C. P. Schwalbe and T. M. ODell

This project entails a comparison of the survival and development of F_1 sterile, wild and normal laboratory reared gypsy moths on oak foliage. This report will be used to finalize the foliage rearing study and present results of any other studies which are related to the feasibility of operationalization of the sterile male technique.

In the last Annual Report, rate of development and survival of a wild strain, the standard laboratory strain (NJSS) and three types of F_1s were compared when the were reared on foliage. To determine if foliage rearing had an impact on fertiltiy of these adults, they were outcrossed to normal laboratory reared stock. Also, to envestage the usefulness of irradiated sterile females (15 Krads) as sentinel females for monitoring F_1 release plots, they wew mated to two types of F_1 males. All egg masses were held for 30 days for embryonation and then held for 180 days at 4-5^{o}C to complete diapause. The results of this mating study are presented on Table 1.

Results are similar to earlier studies which involved making the various types of F_1 crosses when all of the insects were reared on diet (Annual Report, Oct. 1, 1982 - Sept. 30, 1983, GM 8.2.2, pp. 46-57). The mean number of eggs oviposited by normal females, independent of male type, was affected by larval diet. Untreated females, reared on artificial diet (treatments 2, 4, 6, 8 and 10), tended to oviposit greater numbers of eggs. NJSS females (standard lab strain) reared on foliage (treatment 1) oviposited more eggs than either of the two wild (P-1) strains reared in the same manner (Treatments 3 and 5). Similar to earlier studies, F_1 females, whose male parent received the highest dose of radiation (10 Krads - Treatment 11), tend to produce fewer eggs than F_1 progeny of males treated with lower doses.

The degree of embryonation of the various types of crosses was also similar to results of previous studies. Egg masses produced by F_1 males x untreated females have a lower percentage of embryonation than when either both parents are normal or when the male is untreated and the female is an F_1. Egg masses having the smallest proportion of eggs embryonated were produced by F_1 males whose male parent received the intermediate dose of radiation. This result is unexplained, but is consistent with earlier studies. F_1 females, mated to normal males (similar to mating where both parents are normal) produce egg masses with large proportions of the eggs embryonated.

In the three treatments (12, 13 and 14), where irradiated females were crossed with F_1 males, embryonation was so low that determining if the female was mated would be impossible. Irradiated females, at least at this treatment level, therefore, are unsuitable for monitoring sterile male treatments.

Unfortunately, hatch from all of the treatments was much lower than expected. Egg masses from the control treatment (Treatment 1) had only 29% of the embryonated eggs hatch. The poor hatch results are unexplained because eggs were held under standard embryonation and diapause conditions.

Table 1. Fertility of wild, laboratory and F_1 strains of gypsy moth when reared on foliage and outcrossed to laboratory reared adults.

Treatment Number	Mating Type Male	X Female	No. of Mating Pairs	Mean No. of Eggs/ Mass	Mean Percent Embryonation	Mean Percent Embryonated Eggs Hatched	Mean Percent of Total Eggs Hatched
1	NJSS	NJSS	19	608.3	81.7	29.1	25.7
2	Wild (Upton)	Lab*	9	863.1	79.9	45.9	34.4
3	Lab*	Wild (Upton)	10	372.9	92.5	27.7	25.1
4	Wild (PA)	Lab*	5	775.8	85.0	44.8	36.1
5	Lab	Wild (PA)	7	153.0	71.7	53.5	34.2
6	F-1 (6 Krad)	Lab*	10	328.4	40.6	25.3	6.2
7	Lab*	F-1 (6 Krad)	8	659.5	84.2	7.4	6.0
8	F-1 (8 Krad)	Lab*	11	585.8	22.1	0.0	0.0
9	Lab*	F-1 (8 Krad)	8	150.6	82.7	0.0	0.0
10	F-1 (10 Krad)	Lab*	10	446.7	40.6	18.4	10.3
11	Lab*	F-1 (10 Krad)	9	323.8	83.4	2.9	2.2
12	F-1 (10 Krad)	15 Krad*	23	325.8	0.0	0.0	0.0
13	F-1 (8 Krad)	15 Krad*	20	386.5	1.8	5.0	0.1
14	F-1 (6 Krad)	15 Krad*	48	390.4	0.1	0.0	0.0

* Reared on artificial diet under laboratory conditions.

Project Number: GM 4.2.1
Project Title: Suppression of Gypsy Moth Populations with Releases of F_1 Sterile Eggs - Maryland
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, T. M. ODell

Egg mass evaluation for determination of type (F_1 or wild) of insects collected in the two Maryland treatment plots has been completed. The data will be analyzed and a final report submitted in the next Annual Report.

Project Number: GM 4.2.2
Project Title: Evaluation of Competitivness of F_1 Sterile Neonates: A Round Robin in the Square Forest.
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, J. D. Tang and C. P. Schwalbe

This study was initiated in the 1984 field season to evaluate the ability of F_1 sterile neonates to move off of eggs and, subsequently, locate and climb vertical objects (ie. locate and climb host trees). Results from the 1984 studies were inconsistant and not conclusive. There was an indication that a larger proportion of F_1 sterile larvae failed to leave the eggs after hatch but, generally, there were no gross differences between progeny of wild insects or laboratory insects (F_1 steriles or normals) detected.

In 1985, we attempted to repeat and improve these tests. Test design was similar to 1984, however, to correct the problems associated with volumetric estimates of numbers of eggs, all eggs were counted. For testing, petri dishes containing known numbers of eggs were placed in the center of circular plots, early on the first morning of the test. Posts (4" x 4" x 4'h), arranged in two concentric circles (3m radius - 6 posts, 6m radius - 12 posts) around the release site were checked every 15 minutes. All neonates found on a post were counted, recorded and removed. At the conclusion of observations on a day (dusk), the top was placed on the petri dishes containing eggs to prevent predation and then removed early the following morning (dawn). At the end of a test, the petri dish was returned to the laboratory, frozen and all remaining eggs and neonates counted.

Because of personnel and time constraints, only two replicates of this test were carried out during the 1985 field season. In the first replicate (June 16 to June 18), F_1 sterile neonates were compared with a wild strain (Ashumet). The timing of these tests was not optimal because temperatures were higher for this period than the normal wild egg hatch period (ca. May 10). The second replicate was conducted between August 28 and 29. Again, temperatures were not comparable to the spring hatch period, and because wild eggs were not available, eggs from the NJSS laboratory strain were used for comparison.

The results of these two trials are presented in Table 1. In both trials, a larger proportion of F_1 neonates left the egg dishes and dispersed. Twenty-seven percent of the wild neonates, and 20% of the NJSS neonates failed to leave the egg dishes. Larger proportions of wild and NJSS neonates however, located and climbed posts. Although a slightly higher proportion of F_1s were found on the outer circle of posts,the fate of the large number of F_1 larvae which dispersed (left the eggs) but did not find posts is not known. Interestingly, these results are almost exactly opposite of the 1984 test results, where a larger proprotion if F_1 larvae failed to leave the dishes, and at least in one test, a larger proportion were captured on posts.

Results of the 1985 trials are inconclusive, and a number of reasons for the apparent difference could be offered. However, we are currently designing a set of laboratory and small field tests to evaluate the ability of neonates to locate hosts. These tests will, initially, be conducted under more controlled conditions and in areas where observational data may offer some behavioral insights into this initial dispersal period.

Table 1. Dispersal of F_1 sterile wild and laboratory (NJSS) neonates in circular plots.

Strain	No. Larvae When Hatched	% Larvae Not Dispersing	% Dispersing Locating Posts	% Recaptured On Outer Circle
		June 16-18, 1985		
Wild (Ashumet)	7073	27.9	76.9	7.2
F_1 (10 Krad)	3922	8.1	16.2	12.5
		August 28-29, 1985		
NJSS	9504	20.1	82.8	4.6
F_1 (10 Krad)	9534	8.6	10.7	8.9

Project Number: GM 5.2.1
Project Title: Bioassay of Gypsy Moth 1983-1985 Disparlure Dispensers
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: E. C. Paszek and V. C. Mastro

Pheromone dispensers are used in traps to survey large areas for gypsy moth infestations. These dispensers are stored in a freezer prior to use and are annually tested in a field bioassay. In the 1984 bioassay of the 1981-1984 dispensers on inventory, there was a significant difference between the 1981-1983 aged dispensers and the unaged dispensers. The aged dispensers outcaptured the unaged dispensers 4 to 1. The 1984 dispensers showed no significant difference between aged and unaged. It was recommended that dispensers probably should be aged for a minimum of a week before they are used, to maximize attractiveness.

Samples of the 1983-1985 dispensers were individually pierced onto common pins and hung on a tack board, on a shaded east wall inside the greenhouse. They were aged for 2 weeks (7/23 - 8/5), 6 weeks (6/25 - 8/5) and 12 weeks (5/14 - 8/5) at temperatures that averaged a low of 26°C and a high of 38°C. The unaged controls were stored in a freezer and a desk drawer at room temperature. They were placed in milk carton traps and 7 replicates were bioassayed in a 14 X 8 grid 50m apart in Yarmouth.

Table 1. Bioassay of aged and unaged 1983-1985 disparlure dispensers on inventory.1/

Year-Lot No.	Mean No. of Moths Caught Weeks Aged			Freezer Unaged	Room Temp Unaged
	2 wks	6 wks	12 wks	0-day	0-day
1985 - 185	71.21abc	81.64ab	43.50 cde		63.57abc
1985 - 175	64.43abc	65.68abc	52.50 bcd	28.36 de	
1984 - D0274	62.11abc	58.71abc	57.46abc	84.00a	
1983 - D0053	58.07abc	79.11ab	47.11 cde	24.36 e	

1/ Treatments followed by same letter not significantly different at the .05 level.

Both lots of the 1985 lure dispensers, aged for 12 weeks, were significantly different than similar samples aged for 2 weeks. The smaller number of moths captured indicates that at 12 weeks of aging at greenhouse temperatures, disparlure begins to dissipate from the Hercon dispenser. The 1985 lot no. 185 unaged sample, stored at room temperature, compared favorably to the 2 and 6 week age samples. The 1985 lot No. 175 unaged sample, stored in a freezer, captured significantly fewer moths that lot no. 185, which was stored at room temperature. Storing lure in a freezer retards its activity for approximately 2 weeks. There were no signficant differences between all of the 3 aged samples of the 1984 lure. However, there was a significantly higher number of moths captured by the unaged 1984 freezer control sample. This sample captured moths immediately and did not require an aging period to reach activity. This 1984 lure sample behaved similarly in the bioassay conducted in 1984. The 1983 and 1985 lures, stored in the freezer, required an initial aging period to become active. This delayed activity does not affect trapping performance when lure dispensers are placed in the field several weeks prior to the anticipated moth flight. However, later in the flight season, when delimiting infestations with additional new traps, it is recommended to use aged lure dispensers in the new traps. The new lure dispensers, used in these traps, should be aged for at least a minimum of one week.

To evaluate two lots of pheromone dispenser from Hercon, a post season field trial was initiated. The first of these test lots, D0665, was formulated as laminate which was enclosed in a "tea bag". The second test lot (L37556) was also formulated as a laminate, but not enclosed in a tea bag.

For testing, traps baited with the two test lots, described above, (D0175 and L37556 and another test lot (D0185) were compared to traps baited with 100ug and 10ug of (+) disparlure (Plimmer-Schwartz standard) dispensed on cotton wicks. Traps were placed on a circular pattern (40m inter-trap spacing X 140m radius) around a central male release station. Traps were arranged so that all treatments were represented in an arc describing one third of the circle and all trap treatments were replicated three times.

Adult males were released from the center of the plot daily. Also, daily traps were checked, cleared of moths and rerandomized. Cotton wicks were replaced every three days. Tests were initiated on September 3 and not completed until October 8. Several tests during this period were delayed or abandoned because temperatures prevented male flight. In all, data from seven readings were used in the analysis.

Table 2. Mean number of male gypsy moths captured in delta traps baited with various (+) disparlure formulations.

Pheromone Treatment	Mean Numbers of Male Gypsy Moths Captured Per Trap 1/
D0665 Laminate - Tea Bag	33.7 a
D0175 Laminate	32.7 a b
L37556 Laminate	24.3 a b
10ug (+) Disparlure cotton wick	25.7 a b
100ug (+) Disparlure cotton wick	23.7 a b
D0185 Laminate	18.7 b
Blank Trap	.3 c

1/ Means followed by the same letter are not significantly different at the 5% level according to Duncans Multiple Range Test. Analysis performed on transformed means [log (n+1)]; actual values presented.

Results of this lure comparison are presented on Table 2. Because of the difficulty in conducting this test (ie. variable results because of weather constraints) these lures should be compared in a 1986 test during the native gypsy moth flight period. However, these preliminary results do not indicate that traps baited with the two test lots (D0665 or L37556) captured numbers of males significantly differently from the 1985 standard (lot D0175). Indeed the number of males captured by traps containing D0175 lure was nearly the same as traps containing lure lot D0665, which contains the same source of lure as D0175 only the laminate is placed in a "tea bag".

Project Number: GM 5.2.2
Project Title: Male Moth Dispersal in the Absence of Pheromone Sources
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: C. P. Schwalbe, E. C. Paszek, V. C. Mastro

These tests were conducted to determine the movement of male moths released into an area devoid of pheromone sources in order to provide some further insight into the effectiveness of detection trapping programs (where populations are very sparse and traps at least 1 mi apart). Some believe that under such conditions moths will fly great distances to trap sites. The experiment was conducted on Cape Cod (Crane Wildlife Preserve and Yarmouth) before native moth flight commenced (June 18 - July 8, 1985). Two plots, each 1 mi^2, were established and gridded with 100 trap locations (ca 530' or 161 m between grid points). On each of the first 3 days of each test, 0-1 day old laboratory reared moths were collected, divided into two groups and marked appropriately with Day-Glo powder. One group was released into the center of each plot and the other held in large cages in an outdoor insectary. On the fourth day, milk carton traps were deployed before 8:00 AM and all caged moths were released into the trapping plots. Groups of 0-1 day old males were also released in each plot. All traps were recovered 3 days later and the position in the grids that variously marked individuals were captured was recorded. The test was repeated 3 times. An attempted 4th replicate was aborted due to native male moth occurance.

The design of this experiment provided for moths to age, disperse and suffer mortality for 1, 2, and 3 days prior to trap placement. Additionally, the isolated effect of age on capture was tested by releasing males (after traps were deployed) aged in cages for 0, 1, 2 or 3 days. Results are tabulated in Table 1. Capture of males released directly into the grid of traps was low (2.21 to 5.56%), but consistent with previous experiments conducted here and elsewhere (Michigan State University, University of Massachusetts). Certainly, avian predation accounted for part of this loss as birds were seen feeding at the center release sites. In fact, it appeared that birds rapidly moved into the area when releases were made. Nevertheless, most moths that were released into the grid of traps were caught in traps closest to the release site ($\bar{x}$ = 130 - 217 m). Moth age at the time of release did not have a profound effect on capture rates, although % capture was greatest for moths 2-3 days old at release.

Capture rates for moths released 1-3 days prior to trap placement was decidedly lower (0.83 - 1.43%) indicating considerable initial mortality of moths within 24 hours of release. In this case, however, fewer moths were recovered close to the release site and mean distance from point of release to point of capture was 347-495 m. These results suggest that moths tend to fly farther from the release point in the absence of pheromone. Most clearly, however, capture (survival?) near the release area drops off very quickly following release.

Table 1. Total number of moths captured various distances from a central release point in plots with traps placed 161m apart. Test was replicated 3 times in 2 plots (June 18 - July 8, 1985).

Day Moths Collected	Day Moths Released	Day Traps Placed	Trap Distance from Release Site (m)														Σ	# Released	% Captured	$\bar{x}$ Meters to Capture Site
			111	249	337	406	463	565	607	687	725	763	800	830	918	1028				
1	4	4	22	12	1	2	4			1							42	960	4.38	217
2	4	4	18	11	2	3							1				35	630	5.56	212
3	4	4	17	1	1												19	860	2.21	130
4	4	4	25	8	2	1	2										38	1090	3.49	178
1	1	4	1	1	2	2	2										8	960	0.83	347
2	2	4		2		1	2	1		3							9	630	1.43	495
3	3	4	2	1	2	1			2					1		1	10	860	1.16	462

Project Number: GM 5.2.3
Project Title: Sterile F_1 Demonstration Project - Bellingham, WA.
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Report Leaders: V. C. Mastro, C. P. Schwalbe, J. D. Tang and D. R. Lance.
Cooperator: Eric Lagasa, Washington State Department of Agriculture

To demonstrate the feasibility of eradicating isolated infestations of gypsy moth with releases of F_1 egg masses (progeny of males treated with 10 Krads x untreated females), a release was made into a population in Bellingham, WA in 1985. This population was detected in 1983, when 15 males were captured in pheromone-baited traps. Based on the results of the 1983 trapping (not a delimitation grid), two small areas were ground treated with insecticide and a larger area was mass trapped. However, adult trapping in 1984 revealed that most of the infested area was to the north and east of the mass trapped and sprayed areas. A total of 82 males were captured in 1984, 77 of these outside of the 1984 treatment areas and in the area which was used in 1985 for the F_1 egg release study. In the 1984 delimitation trapping program, traps were not placed on a uniform grid system; inter-trap spacing however, averaged ca 131 m. At this trap density, approximately 20% of all males should have been captured, or a total of 385-400 males should have been present in 1984. Egg mass scouting during the winter of 1984 located a total of 9 wild egg masses, oviposited in 1984, and several old egg masses and larval and pupal skins.

Based on the distribution of male trap catches in 1984, boundaries of the area for treatment in 1985 were established. Also based on the 1984 trapping data, an estimate of overwintering egg mass population was calculated (ie. assuming a 1984 population of 400 males and a 1:1 sex ratio, 400 females and 400 overwintering egg masses would be present in 1985). To determine the number of F_1 egg masses to be released in 1985, several factors were taken into consideration. Ideally, if eradication is to be achieved in the shortest possible time using the sterile male technique, the sterile:fertile overflooding ratio should be as large as possible. If males are competitive, 100 sterile:1 fertile (100S:1F) adult male ratio would result in 1 fertile mating per 101 matings. Given no other constraints, we would have attempted to release a large enough number of F_1 eggs to provide adult ratios as large or larger than 100S:1F. The area, however, was highly residential and there was not an abundance of host trees within the area. Therefore, we chose to lower the numbers of F_1 initially released and target a lower overflooding ratio.

Methods and Materials:

On April 3, approximately 34,000 F_1 egg masses were released. Characteristics of F_1 egg masses are that they have a lower percentage of hatch than normal egg masses and a lower larval survival rate. Therefore, 34,000 F_1 egg masses are equivalent, in terms of numbers of adults produced, to 13,600 normal egg masses. It was calculated that the release of this number of F_1 egg masses would produce a 34S:1F male overflooding ratio (i.e. 13,600 F_1:400 wild egg masses).

To distribute egg masses, the entire target area was surveyed on a property by property basis (221 properties). Based on the number and size of host trees on a property, an estimate was made of the number of F_1 egg masses which could be placed without causing defoliation. In actual egg mass placement, 160 properties were treated. During treatment, individual trees were evaluated for their ability to support gypsy moth larvae and an appropriate number if egg masses was dispensed around the base of the tree.

To evaluate the percentage and synchrony of hatch of F_1 and wild egg masses, 9 wild egg masses and 27 F_1 egg masses (3 from each of the 9 egg mass production lots of eggs released in Bellingham) were placed in a cage designed to prevent escape but which provided normal ambient temperature conditions. Egg masses were monitored daily and all newly hatched larvae were removed and recorded.

To compare larval establishment and development and to track sterile:wild male overflooding ratios, collections were made throughout the larval and pupal stages. To facilitate larval collections, trees throughout the treatment area were burlap banded (Table 1). To collect a representative sample, 6 sites (properties) were randomly selected daily and 5 insects were collected from each property (e.g. 30 insects a day). Larvae were individually placed in 1-1/2 ounce cups containing diet (labled with collection date, location and host) and returned to the Otis laboratory for evaluation. Pupae were treated in a similar manner except cups were packed with tissue paper to prevent damage in transit. Immatures received at the Otis laboratory were held for development under standard rearing conditions.

Male larvae from these collections were evaluated using two methods. All male larvae (1st through 4th instar) were divided into two groups. The first group was used for chromosome analysis to determine if the males were wild (31 pairs of chromosomes - no translocations) or F_1 steriles (not 31 pairs of chromosomes and visible translocations). The technique used for chromosome analysis follows: Testes were removed from male larvae from the period beginning 4 days after the 3rd/4th larval ecdysis and ending one day after the 4th/5th instar ecdysis. The testes were then placed in a drop of saline solution on a glass slide and the spermatocytes were teased from the peritoneal sheath. A drop of orcein stain (4 gm orcein, 80 ml acetic acid and 80 ml distilled water) was added to the spermatocytes after the sheath and any other connective tissue was removed. A cover slip was then placed on the slide and squashed firmly. After allowing time for the spermatocytes to stain, the slide was examined under a compound microscope (at magnifications of 400 and 650x) for the number of pairs of chromosomes and the presence of translocations.

The other group of the males collected as larvae between the 1st and 4th instars and all male 5th instar larvae and pupae were reared to the adult stage and individually mated to normal laboratory females. After mating, resulting egg masses were allowed to embryonate for 30 days and then dehaired and the percentage of embryonated eggs determined. The two possible types of crosses produce dissimilar types of egg masses; F_1 males x normal females produce egg masses with a low proportion of embryonated eggs ($\bar{x}$ = 28%) while normal male x normal female crosses produce egg masses with high proportions of embryonated eggs ($\bar{x}$ = 94%).

All females collected as either larvae or pupae were individually reared to the adult stage and mated to normal laboratory males. To distinguish F_1 females from wild females, it is necessary to hold the egg masses throughout the embryonation and diapause periods and base the evaluation on hatch. Embryonation of egg masses produced by F_1 female x normal male crosses is 70% vs. 94% for normal female x normal male crosses. However, hatch of embryonated eggs for the two types of crosses are dissimilar, 0.7% and 76.4% respectively.

Overflooding ratios in the adult stage were monitored by, daily, placing one day old virgin F_1 females (progeny of males treated with 10 Krad and x untreated females) within the release area. Females were placed early in the morning and retrieved in the evening. To discourage predators, females were placed in modified delta traps. The traps used were folded so that the end panels remained open and the trap was lined with burlap (no tack trap) to provide a substrate for females to grip. After retrieval, females were returned to the laboratory and allowed to oviposit. After embryonation was complete, egg masses were examined and a determination of male mating type was based on the percentage of eggs in the masses which were embryonated (ie. F_1 male x F_1 female crosses produce egg masses with ca. 10% of the eggs embryonated vs normal male x F_1 female crosses which produced egg masses with ca. 70% of the eggs embryonated).

After all adult flight was over, egg masses were collected from under burlap bands (64 total) and are being held for a determination of mating type:

1) F_1 male x wild female
2) F_1 male x F_1 female
3) Wild male x wild female
4) Wild male x F_1 female.

In addition, an egg mass search will be conducted in the late winter to locate more egg masses for evaluation.

To monitor the male flight period and to determine if the treatment area was isolated from any other possible infestations, a nine square mile area, centered over the F_1 release area, was trapped at the rate of 36 USDA milk carton traps per square mile. Traps within and adjacent to the release area were checked daily; traps located within two grid lines of the release area were checked weekly and the remaining traps were checked twice during the season.

Results:

Hatch of wild egg masses produced a mean of 279 neonates per egg mass, while hatch of F_1 egg masses produced a mean of 162 neonates. Hatch of wild and F_1 caged egg masses in Bellingham, WA was not in perfect synchrony (Figure 1). Egg masses released in Bellingham were from several production lots which displayed different hatch characteristics. As expected, some of these lots began hatch sooner because temperatures were high. After a small hatch peak for F_1 egg masses (on Julian date 105), hatch for wild eggs began. Temperatures for Julian dates 102, 103, 104 and 105 were, respectively, 62, 59, 58 and 70°F. However, temperatures began to drop (ie. the maximum temperature for Julian dates 106 through 112 was 53°F) and hatch rates for both types of egg masses slowed. Peak hatch from both types of egg masses occurred on Julian date 117 and again on Julian date 123. These two peaks cannot be explained by daily temperature fluctuation (ie. maximum temperatures for Julian dates 113, 114, 115, 116 and 117 were, respectively, 55°, 51°, 50°, 50° and 52°F) but probably are a function of accumulated degree days.

In total, 2650 gypsy moth larvae and pupae were collected from within the Bellingham release site. Of these, 538 male larvae were dissected for chromosome evaluation. Four hundred and fifty-eight of those dissected were determined to be F_1 males (translocations present) and 8 were determined to be wild males (31 normal chromosome pairs). This evaluation provides an estimate of the S:F overflooding ratio of 57.3:1 for insects collected as first through late fourth instars. No determination of type (sterile or fertile) could be made on 72 male larvae that were dissected (e.g. dissected at the wrong stage, poor slide preparation, no visible chromosomes, etc). In addition, 159 female larvae were mistakenly identified as male larvae and dissected and no determination of type can be made on these. Errors in judging sex or age of larvae decreased as the season progressed and as the technique became routine. Experimental work in 1983 and 1984 demonstrated that a slightly higher proportion of F_1 larvae die in the early instars. Therefore, overflooding ratios based on dissections of later instars should provide a better estimate of the eventual adult overflooding ratio. If only insects, collected after May 29 (Julian date 149) and dissected (total of 262 male larvae), are included in the calculations, the overflooding ratio is 32S:1F. May 29 was chosen because larval development records indicate that on May 30 (Julian date 150), the first fourth instar larvae was collected (Figure 2).

In all, 693 males were reared to the adult stage and mated to normal laboratory reared females. Evaluation of egg masses after the 30 day embryonation period determined that 677 of those males were F_1 steriles and 11 were wild fertiles. For the remaining 5 mating pairs, no determination could be made of male type. The calculated season-long S:F overflooding ratio from this mating data is 62S:1F. If only males collected on May 30 or after are considered (n=483), the computed overflooding ratio is approximately 80 S:1 F. Collection locations where males which were later determined to be wild, either through chromosome analysis or mating, were widely scattered throughout the release area (Table 2).

Egg masses resulting from females collected as immatures and mated to normal males and, also, egg masses collected throughout the release area are now being evaluated. Results will be reported in the 1986 Annual Report. The evaluation of egg masses oviposited by monitor F_1 females is now complete. A preliminary tabulation indicates that, of the 150 egg masses evaluated, 12.7% are the result of matings with F_1 males. None were the result of matings with wild males and the remaining 87.3% were either the result of F_1 male matings or the female did not mate (ie. the egg masses contained all unembryonated eggs).

Of interest was the relatively high parastism rates observed in the insects which were collected as larvae. A braconid parasite, Cotesia melanoscelus (Ratz), emerged from 201 of the larvae (ca. 9.9% of all immatures collected) and a tachinid parasite, Compsilura concinnata (Meigen) emerged from 220 larvae (ca. 10.9%).

The pattern of male trap catches, within the nine square mile area, indicated that the area treated with F_1 eggs was isolated from any other gypsy moth infestation. The heaviest concentration of males were captured within the boundaries of the release area (a total of 745 males). Most other males were captured 1 or 2 grid lines adjacent to the release area (a total of 9 males). There was no pattern of multiple trap catches outside of the release area which would have indicated that a pocket of infestation was overlooked and left untreated. Since dead F_1 males cannot be distinguished from native males, these data are not useful for estimating overflooding ratios.

Table 1. Trees burlap banded for larval sampling, Bellingham, WA, 1985.

Tree Type	Number of Trees
Apple	252
Cherry	143
Birch	61
Maple	41
Oak	36
Hazelnut	30
Ash	20
Willow	14
Plum	14
Alder	14
Locust	10
Walnut	10
Douglas Fir	10
Laburnum	10
Crabapple	9
Elm	8
Dogwood	8
Hawthorn	7
Viburnum	7
Black Cottonwood	7
Horse Chestnut	6
Sumac	5
Peach	2
Pear	2
Pine	2
Sycamore	2
Dawn Redwood	2
Lombardy Poplar	2
American Linden	1
Cascara	1
Tulip Tree	1
Western Hemlock	1
Catalpa	1
Quince	1
Total	740

Table 2. Location of collection sites of immature male larvae which were determined to be fertile through chromosome analysis or mating (egg mass evaluation).

Location	Julian Date of Collection	Number of Larvae
Determined by Chromosome Analysis:		
3011 Elm Street	164	4
3021 Kulshan Street	175	2
3000 Elizabeth Street	175	1
1700 Maplewood	176	1
	Total	8
Determined by Mating:		
2931 Elizabeth Street	113	2
1310 Oregon Street	123	2
1302 Maplewood Street	140	1
3011 Elm Street	164	2
2931 Elizabeth Street	170	1
Behind Mall Area	191	1
1311 Maplewood Street	197	1
3021 Kulshan Street	200	1
	Total	11

The data from the Bellingham pilot project indicate that the release of F_1 eggs was successful in establishing an F_1 sterile population which interacted with the native population. Furthermore, monitoring of sterile:fertile ratios, using the two techniques (chromosome and mating evaluations), indicates that the target overflooding ratio was achieved. Because a lower overflooding was selected, and apparently achieved, eradication of the population would not be expected.

Male trap catches during the 1985 season provide an estimate of the actual male density. In other studies, grids of traps with a similar spacing as used in Bellingham, recovered approximately 9.1% of the males released from a center release point. Although the percentage recovery in Bellingham was probably larger because males were more evenly distributed (not released from a central point), a 9.1% recovery rate would indicate that the total male population was 8,286. If the overflooding ratio of 34S:1F is used to calculate the numbers of wild and F_1s, respectively, there would have been 237 and 8063 males. Assuming a 1:1 sex ratio for the wild insects, 237 wild females would have been present in 1985. Again, using the sterile to fertile overflooding ratio of 34:1 only 7 wild females should have been mated with wild males in 1985. Based on this estimate of wild type matings, to achieve a 100S:1F overflooding ratio in 1986 would require the release of 1750 F_1 sterile egg masses (i.e. 7 x 100 [overflooding rate] x 2.5 [differential hatch and mortality factor]). To ensure eradication in 1986 and to provide a uniform distribution of sterile eggs throughout the release area, a slightly larger number of F_1 egg masses will be released.

Figure 1.

Egg Hatch Synchrony of Wild and F-1 Egg Masses in Bellingham, WA - 1985

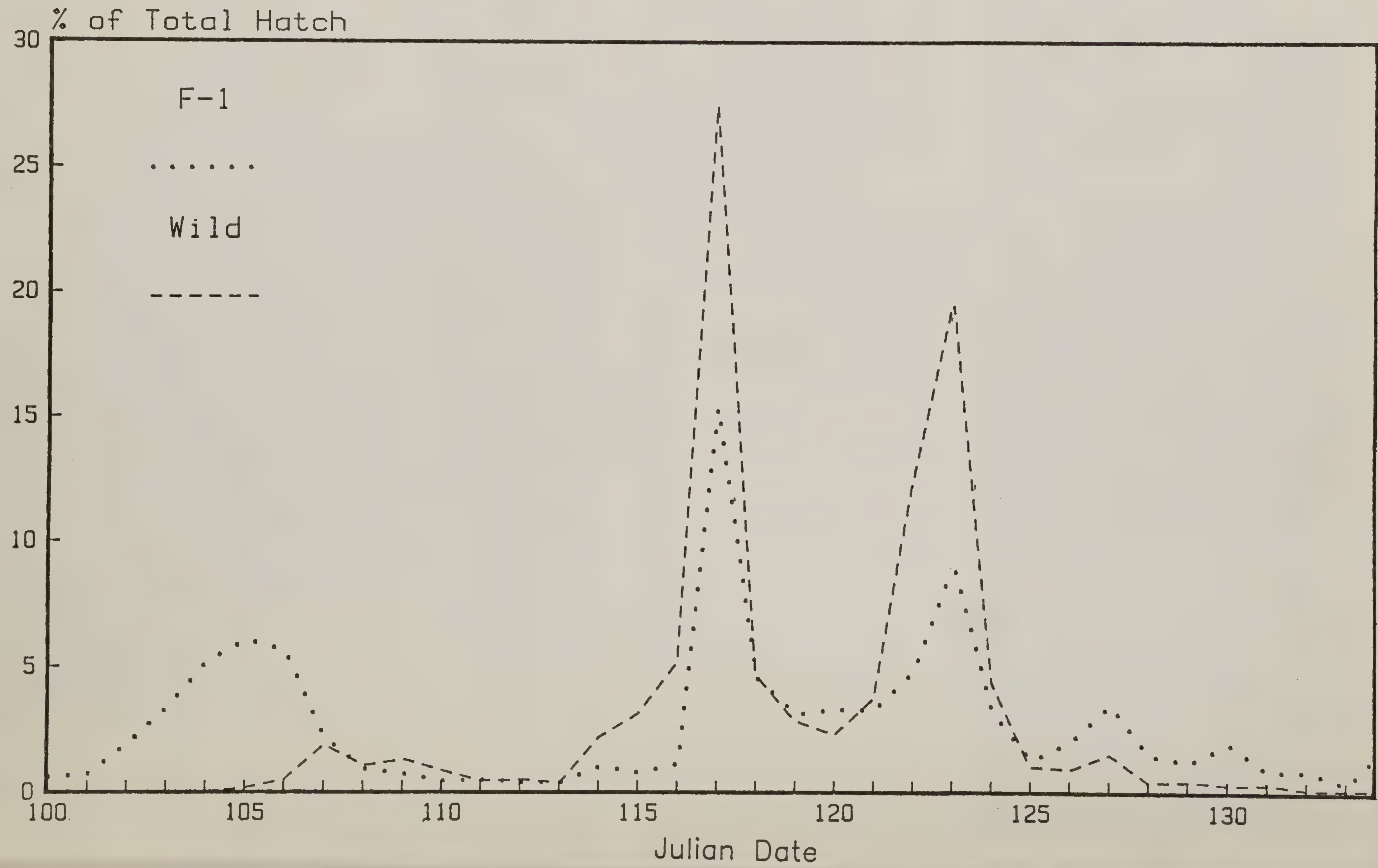
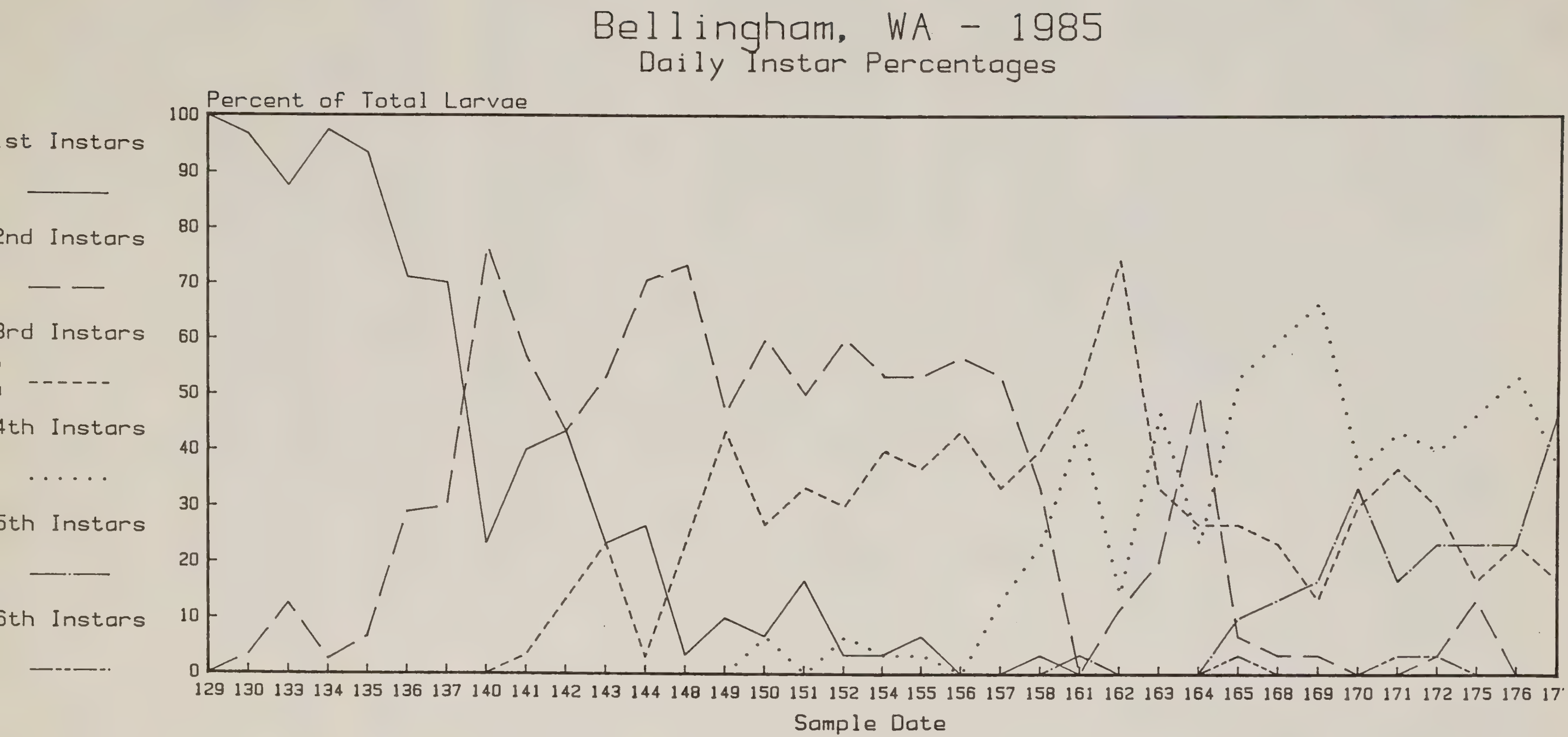


Figure 2.


Bellingham, WA - 1985
Daily Instar Percentages
Percent of Total Larvae
1st Instars
2nd Instars
3rd Instars
4th Instars
5th Instars
6th Instars
100
90
80
70
60
50
40
30
20
10
0
129 130 133 134 135 136 137 140 141 142 143 144 148 149 150 151 152 154 155 156 157 158 161 162 163 164 165 168 169 170 171 172 175 176 17'
Sample Date

Project Number: GM 5.2.4
Project Title: Sterile F_1 Demonstration Project - Darke Co., Ohio
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: V. C. Mastro, C. P. Schwalbe, J. D. Tang and D. R. Lance
Cooperators: Ken Roach, Ohio Department of Agriculture

Introduction:

The objective of the project was to demonstrate the feasibility of eradicating an isolated population of gypsy moth using the F_1 sterile egg release technique. An isolated, sparse population in Darke Co., Ohio was chosen as one of the two sites for this type of demonstration (see GM 5.2.3, Sterile F_1 Demonstration Project Bellingham, WA). Techniques and procedures used in this test were generally similar to the Bellingham project. Only when there are differences, will they be described in this report.

The population of gypsy moth selected for treatment was first detected in 1982. In 1984 when the area was delimitation trapped, a total of 97 males were captured. Subsequent egg mass scouting confirmed that the infestation was in a roadside park and possibly in an adjacent area which contained a trailer park and restaurant. A total of 27 egg masses were located. All of these were on shagbark hickory trees within the trailer park.

Because the trap spacing used for delimitation in 1984 was not uniform and because some 1984 traps were placed after flight had begun, an estimate could not be made of the native adult gypsy moth population using trapping data. However, an estimate of density was made based on the numbers of native egg masses found. It was assumed that, given the intensity of the egg mass survey, the 27 egg masses found represented 20% of all native egg masses or 135 egg masses should have been present in the spring of 1985. To achieve an adult male overflooding of 100 sterile:1 fertile (100S:1F), it was calculated that 33,750 F_1 egg masses would need to be released.

Methods and Materials:

The area considered infested (11 ha) was surrounded by agricultural fields. On April 17, F_1 egg masses were released by scattering them along lines (ca 25m apart) within the woods surrounding the roadside park. Within the park area and in the trailer park and restaurant areas, eggs were scattered around individual host trees.

To monitor egg hatch, samples of eggs from different production lots (Julian dates 305, 306, 307, 309, 310, 312, 320, 321 and 322) were caged with five wild egg masses and checked daily.

Similar to the Bellingham demonstration project, daily larval and pupal samples were collected randomly, from throughout the release area. Also during the adult flight period, monitor females were placed and retrieved daily. A nine square mile area (centered on the release site) was trapped at the rate of 36 traps/sq. mile to monitor male flight and determine the degree of isolation.

Results:

Synchrony of hatch of wild and F_1 eggs could not be compared because five wild egg masses failed to hatch. The probable cause for the poor viability of these egg masses was overwintering mortality. Several sub-zero days occured during the winter of 1984-85. Microscopic examination of the eggs confirmed that they were embryonated. During the egg mass survey, all the wild egg masses which were located, were high on the boles of trees where they would be the most susceptable to cold temperatures. Samples of caged F_1 egg masses (n=21) produced hatch over a period of 14 days. However, peak hatch occured for all samples on April 22.

Larval collections began on May 8. On this date larvae were predominately 1st and 2nd instars but ca 5% were in the 3rd instar. Larval development for the site is summarized in Figure 1. A total of 1,823 immature gypsy moths were collected from within the release site. Of these, a total of 222 males were dissected for chromosome analysis. Examination revealed that 187 males were sterile F_1s and 1 was a fertile (wild) individual. There were also 34 males dissected which could not be classified as either F_1 or wild. In addition, 54 females were mistakenly dissected. The one larva determined to be wild, was collected as a 4th instar on May 20th, near the site where all of the wild egg masses were located.

Seven hundred and ninety-seven immature males were reared to the adult stage and mated with normal females for determination of sterility. Predominately, the matings produced egg masses characteristic of F_1 male x normal female crosses (791). Only 2 of the egg masses displayed characteristics of normal male x normal female crosses. Mating type for the remaining 4 egg masses could not be determined. The two fertile type egg masses were from males collected within the trailer park portion of the site (site of 1984 wild egg mass finds). One was collected June 1, and the other on June 10.

Based on chromosone analysis and mating evaluation, S:F overflooding ratios were high: 187S:1F or 395S:1F, respectively. We initially estimated that, given the number of F_1 releases and the estimate of wild egg masses, the overflooding ratio would be 100 sterile:1 fertile in the adult stage. Undoubtedly, the observed overwintering mortality in wild egg masses decreased the native gypsy moth density and could partially explain the higher than expected overflooding ratios.

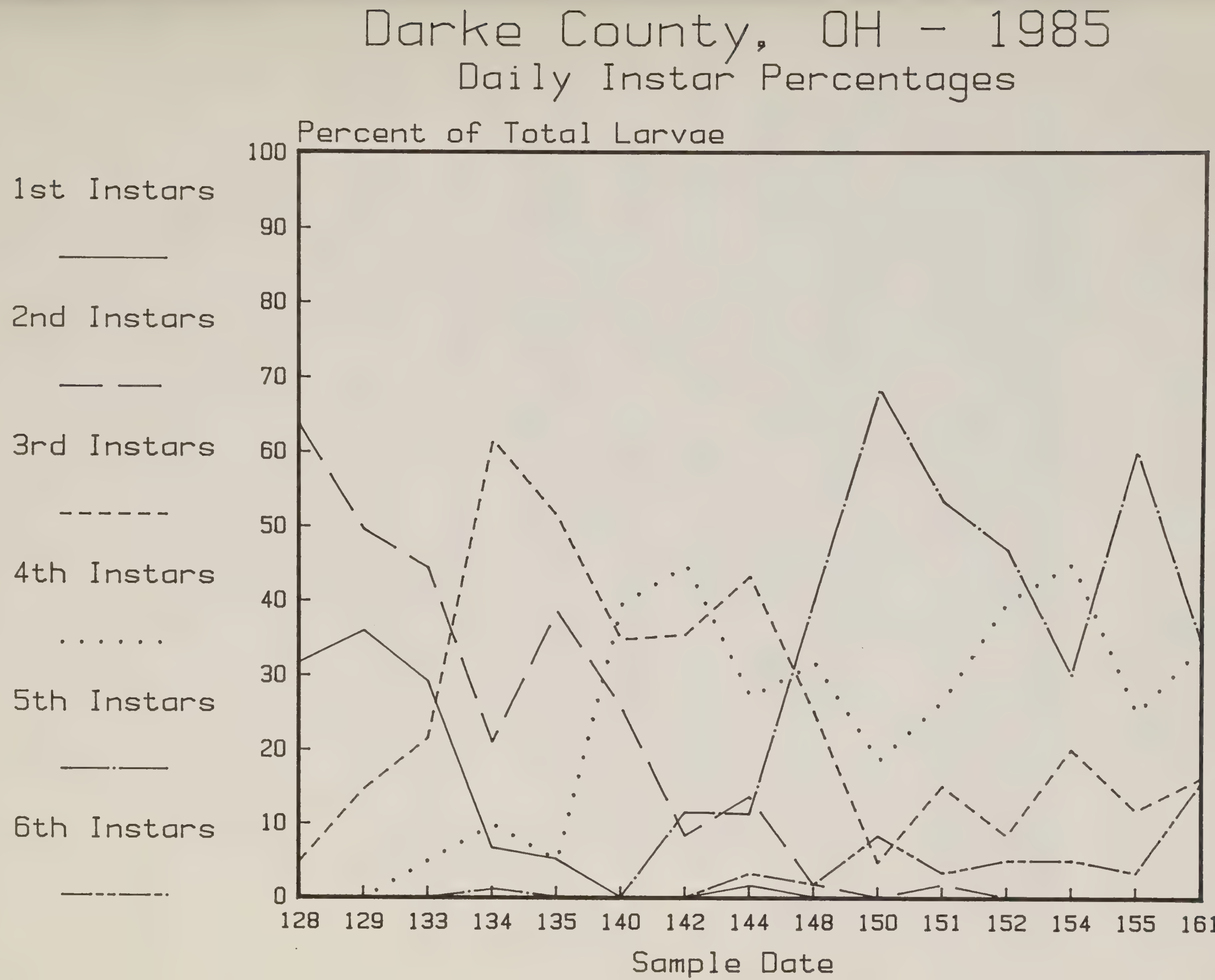
Evaluation of egg masses from monitor females has not been completed. Three hundred and thirty-one egg masses were available for mating type determinations.

Results of the trapping survey conducted over a nine square mile area (36 milk carton traps/sq. mi.) indicate that the native population was isolated. A total of 573 males were captured. The first male was captured on June 27 and the last on August 13.

When most of the insects were in the late larval instar or pupal stages, large numbers of them disappeared from under bands of trees. Several observations of avian predators indicated that this was the main cause of mortality. An invertebrate predator, *Calosoma sycophanta*, was also observed feeding on larvae under a number of burlap bands. Only one parasite was recovered from all of the immature stages of gypsy moth collected. It was identified as an *Exorista* spp.

All of the data analyzed to date indicate that the sterile to fertile overflooding achieved was high enough to expect eradication. This site will only be monitored in 1986 using a 36 trap/mi^2 spacing.

Figure 1.


Darke County, OH - 1985
Daily Instar Percentages
Percent of Total Larvae
1st Instars
2nd Instars
3rd Instars
4th Instars
5th Instars
6th Instars
100
90
80
70
60
50
40
30
20
10
0
128
129
133
134
135
140
142
144
148
150
151
152
154
155
161
Sample Date

Project Number: GM 5.2.5
Project Title: Impacts of Release of F_1 Sterile Egg Masses Within the Generally Infested Area.
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, T. M. ODell and J. D. Tang

Within the generally infested area, three study sites were treated with F_1 eggs to study larval survival of developmental synchrony of F_1s with wild insects and the impact of an F_1 egg release on a native population. In Vermont, 16.2 ha were treated with ca 110,000 F_1 egg masses. Egg mass survey of the area found the native egg mass density to be ca 15.4 egg masses/ha. This release rate should have provided a 25 sterile:1 fertile male ratio in the adult stage.

In Maryland, three wood lots (8.1, 8.1 and 9.3 ha) were treated with quantities F_1 egg masses which were estimated to provide an adult male overflooding ratio of 25S:1F. Because native egg mass densities in these plots were low, wild egg masses collected from another area were seeded into the wood lots at a rate which would provide for a "wild" egg mass density in all plots of 4.05/ha (ie. 10/ac). Similarly, three control plots were seeded with wild egg masses. In two of the treated plots, egg masses were released by hand, scattering them around the base of host trees. In the third plot, eggs were released by aerial application, using a Cesna Ag-Truck aircraft.

The third site treated was in Barnstable County, MA. Approximately 5.8 ha were treated with a total of 118,000 F_1 egg masses. In this site, larval and pupal samples were collected for comparisons of developmental synchrony and the relative survival of F_1 and wild insects. The adult male sterile:fertile mating ratios were monitored daily by placing sentinel females throughout the site. In addition, mating periodicity of F_1 and wild males was monitored by placing one-day old virgin females at sites and replacing them as soon as they mated.

Results from all three of these study sites are now being tabulated and will be reported in the next annual report.

Project Number:	GM 5.2.6
Project Title:	Simplified Methods for Installing Pheromone Dispensers and Vapona Strips in Large Capacity Traps
Report Period:	October 1, 1984 - September 30, 1985
Report Type:	Final
Project Leader:	E.C. Paszek

A simplified method of installing the killing agent and pheromone dispenser inside the milk carton trap was tested. In the standard assembly of the trap, the 4 x 1" laminated killing agent strip was stapled to one end of a Twist'em plant tie. The 1 x 1/8" laminated pheromone dispenser strip was stapled to the plant tie 2 1/2" above the killing agent. The top of the plant tie was bent 1" over the top center of the trap and stapled in place. To simplify this procedure, the killing agent and pheromone dispenser were stapled inside the trap eliminating the plant tie. This was done with a plier-type stapler by directly stapling the killing agent strip below the creaseline, next to the corner, away from the entry ports to the inside of the trap, on the pour spout side. The pheromone dispenser strip was stapled to the inside of the top of the trap in the upper part of the triangle, opposite the pour spout. When the top of the trap is folded together, the pheromone dispenser strip is positioned centrally above the entry ports. A test comparing 15 traps of each of the two designs was conducted in Yarmouth with the following results:

Table 1. Number of moths captured in Standard Milk Carton traps assembled with two variations of pheromone dispenser and killing agent positioning.

Method of Assembly	Number of Moths Captured
Suspended on Plant Twist'em Tie	269
Stapled directly to inside of trap	298

There is no significant difference in moth capture between the two methods of assembling the milk carton trap. The simplified method of directly stapling (with a plier type stapler) the pheromone dispenser and killing agent inside the trap is recommended for assembly of the milk carton traps.

Project Number: GM 7.3.6
Project Title: Insect Production and Distribution
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: J. J. Baker and J. A. Tanner

The primary objectives of the rearing facility are to produce sufficient quantities of all gypsy moth life stages for the support of projects at this laboratory and at research institutions in the United States and abroad, and to produce sufficient quantities of gypsy moth eggs for future projects.

This year, we completed over 1,080,500 matings for the F_1 sterile male program which ran from July 29, 1985 to January 13, 1986. All of the egg masses are presently in storage.

Table 1. The number of eggs, cups and liters of B-4 diet used to provide gypsy moth life stages for in-house and cooperative programs, FY 1985.

Program	Eggs Infested	Neonates Infested	B-4 Diet (Liters)	Number of Cups 6 oz.	Number of Cups 1.5 oz.
ARS			3,888	45,750	
Cooperative Programs	14,880		346	3,122	
Colony	212,754	197,620	3,693	43,448	
Insecticide	1,018,272		1,080	21,214	500
Irradiation Study and Sterile Male QC Testing	1,347,024		7,977	84,189	20,532
Monitoring F_1 Sterile Male	929,360		2,245	26,420	
F_1 Sterile Male					
Plain Diet	3,268,800		19,278	226,800	
Red Diet	544,320		2,891	34,020	
F_1 Wild Strains		156,900	1,207	14,207	
Male Dispersal and Trap Testing	62,880		331	3,900	
Totals	7,398,290	354,520	42,936	503,070	21,032

Table 2. Distribution of reared insects, FY 1985.

Project	Egg Masses	L-2	L-5 L-6	Male Prepupae	Male Pupae	Female Prepupae	Female Pupae
W. McLane (APHIS)		139,300					
V. Mastro (APHIS)				169,796		61,489	
C. Schwalbe (APHIS)				75	11,263	1,120	
R. Carde' (U. Mass)	216						
P. Barbosa (U. Maryland)	520				600		600
C. Yin (U. Mass)	210						
R. Chianese (New Jersey)	4,650						
L. Rhoads (Pennsylvania)	80						
W. Yendol (Penn. State)	490						
G. Carrol (Oregon Univ.)	10						
G. Prestwich (New York)			640				
G. Rosenberry (Maryland)	80						
D. Soper (Ithaca, NY)	25						
J. Tate (Richmond, VA)	55						
T. Kestner (Maryland)					2,650		4,950
Totals	6,336	139,300	640	169,871	14,513	62,609	5,550

Table 3. Summary of sterile male and wild pupal production, FY 1985.

Program		Male Pupae	Female Pupae
F_1 Sterile Male			
Plain		1,087,700	994,630
Red		133,200	115,300
Ashumet Wild		14,591	7,181
Main Wild		331,454	4,574
	Totals:	1,566,945	1,121,685

Table 4. Number of colony eggs produced in FY 1985.

Year	Number of Eggs
1985	76,080,900

Project Number:	GM 1.3.1
Project Title:	Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period:	October 1, 1984 - September 30, 1985
Report Type:	Interim
Project Leaders:	J. A. Tanner and J. J. Baker

The purpose of this project is to monitor the development of the laboratory strains and to determine if it falls within acceptable ranges. Data are collected for each strain/generation and are used to detect changes from normal development so that corrective action can be taken.

Budgetary restraints have forced us to limit collection of developmental data to those insects used to maintain each strain (colony). Currently, only the New Jersey strain is being maintained, but in the near future, we plan to add several wild or near wild strains.

Developmental data are collected only on larval development, female pupal weights, sex ratio, pupal yields, adult female emergence and deformity and female fecundity. The data are collected using the methods described by Tanner, et al. in the 1983 and 1984 Annual Reports.

The yield of colony eggs/colony mating was also calculated. This gives a reasonable estimate of the fecundity of the colony. It reflects not only the ability of the adults to emerge and mate, but also the number of eggs the females can deposit.

Larval "straggling" or "stunting" is a major problem in our rearing program. The condition is characterized by the lack of growth in some newly hatched larvae. An individual rearing cup may contain early, mid- and late-stage larvae as well as pupae. At times, the reduction in pupal yields may exceed 40% (personal observation). Those larvae in the early or mid stage never reach the pupal stage unless they are transferred to fresh diet. Electron microscopic examination of the stunted larvae has shown that these larvae contain a Rickettsia-like organism (RLO) in addition to nuclear and cytoplasmic polyhedrosis virus (Adams, personal communication).

Observations on the growth rate of larvae from individual egg masses indicated that the egg masses can be classified into distinct groups, based on the distribution of the larvae in the first 4 instars, 11 days after egg incubation. One group of masses produced larvae that were predominantly in the first instar. The mean larval instar (MLI) (Tanner, et al., 1983), was between 1.00 and 1.25. Most of the first instar larvae had no signs of feeding or growth. There was a considerable amount of silk within the rearing cup and the larvae were often found wandering about the cup and/or trapped between the lid and the cup. These larvae would have been called "stragglers".

A second group of masses produced larvae that were evenly divided between the first and second instar. The MLI was between 1.25 and 1.75. Most of the first instar larvae showed signs of feeding and growth.

A third group of masses produced larvae that were predominantly in the second instar, with only a few first and third instar larvae. The MLI was between 1.75 and 2.25.

A fourth group of masses produced larvae that were evenly divided between the second and third instar. The MLI was between 2.25 and 2.75.

The last group of masses produced larvae that were predominantly in the third instar, with only a few second instar larvae and, on rare occasions, one or two fourth instar larvae. The MLI was between 2.75 and 3.00.

Currently, we do not have a diagnostic test to determine if the different growth patterns reflect different concentrations of RLO, CPV and/or NPV. However, we do feel that if we can select masses that produce higher MLI's, we could reduce, or possibly eliminate, the "straggling" problem.

The MLI's are determined for 100 egg masses by taking core samples 21 days prior to their scheduled use for colony maintenance. The core samples are held 11 days in individual rearing containers with 55ml of B-4 diet. A MLI is determined for each core sample. Based on this information, 18 masses with the highest MLI's are selected for use in colony maintenance. Also, 18 masses with the next highest MLI's are selected as backup masses. All 36 masses are incubated 2 days before the newly eclosed neonates are to be transferred (10/cup) to rearing containers with 85ml of diet. Thirty rearing containers are set up for each of the 18 chosen masses (now called families) or any of the back up masses, if needed. Care is taken at this time to ensure that neonates from the 18 families are not mixed. After the pupae are harvested, the families are mixed in such a way as to potentially produce up to 75 different mating combinations.

The performance and reproductive data for New Jersey colony insects are shown in Tables 1 and 2, respectively. F_{27} generation insects were reared under the old egg-infest method, and is presented here to give some comparison for the new neonate-infest-by-family method (F_{28}). The data between October 1, 1984 and March 25, 1985 (F_{27}), were excluded because there were several changes made in the methods used to infest and rear the colony insects before the final neonate-infest-by-family method was adopted. Due to the differences in the methods used for the F_{27} and F_{28}, neither the performance nor the reproductive data were compared statistically.

Female pupal weights were lower in the F_{28} (neonate-infest-by-family method) than in the F_{27} (egg-infest method). However, larval densities/cup and the percentage of neonates that reach the pupal stage was higher in the F_{28} than in the F_{27}. Therefore, the lower female weights in the F_{28} are probably reflecting the increase competition for food and space.

Adult female emergence was lower and female deformity was higher in F_{28} than in the F_{27}. The reproductive data was also lower in the F_{28}, indicating that the females were less fecund than those from the F_{27}.

The data in Tables 1 and 2, for the F_{28}, include the data from a 4 week period where there was extremely low female emergence due to poor handling of the pupae. Table 3 shows the same adult and reproductive data for the F_{28} without those 4 weeks. Eliminating those weeks resulted in a higher percent emergence that was very close to the percentage obtained for the F_{27}. The percent deformity decreased slightly. The percentage of females depositing egg masses likewise increased as did the number of colony eggs per colony mating. However, eliminating those weeks resulted in a drop in the number of eggs deposited per female. This again indicates that the females were less fecund than those from Generation 27. In fact, the females from Generation 28 had the lowest fecundity since we began to take reproduction data in 1978. At this time, the drop in fecundity is not overly critical, but it should be watched carefully in future generations to ensure that it does not continue to deteriorate.

Table 1. Performance data of New Jersey colony insects.

Parameter	Generation 1/ 27 (n=13) $\bar{x}$	SD	28 (n=27) $\bar{x}$	SD
Larvae Per Cup				
10th PEID 2/	8.9	1.1	N/A	
10th PNID 3/	N/A		9.6	1.7
Mean Larval Instar				
10th PEID	1.83	0.27	N/A	
10th PNID	N/A		2.93	0.25
Female Pupal Weights (gm)	2.43	0.14	2.26	0.17
% Survival Neonate to Pupae	72.6	10.8	92.0	8.0
Sex Ratio M:F	1.2:1	0.1	1.0:1	0.1
% Adult Female:				
Emergence	93.7	5.2	86.8	14.9
Deformity	0.5	1.2	4.6	4.8

1/ Generation 27 - Started July 9, 1984 through October 1, 1984
Generation 28 - Started March 25, 1985 through October 2, 1985
2/ PEID = Post Egg Infest Day
3/ PNID = Post Neonate Infest Day

Table 2. Reproductive data of New Jersey colony insects.

	Generation 1/			
	27 (n=13)		28 (n=28)	
	$\bar{x}$	SD	$\bar{x}$	SD
% of Female Depositing Egg Masses	90.1	6.8	77.0	19.5
# Eggs Deposited Per Female	1093	79	971	129.4
# Colony Eggs per Colony Mating	922	42	760	157

1/ Generation 27 - Started July 9, 1984 through October 1, 1984
Generation 28 - Started March 25, 1985 through October 2, 1985

Table 3. The modified adult emergence and reproductive data for generation 28 of New Jersey Colony insects.

	Generation 1/			
	27 (n=13)		28 (n=28)	
	$\bar{x}$	SD	$\bar{x}$	SD
% Adult				
Emergence	93.7	5.2	91.7	6.5
Deformity	0.5	1.2	4.3	4.2
% of Female Depositing Egg Masses	90.1	6.8	81.3	14.5
# Eggs Deposited Per Female	1093	79	948	123
# Colony Eggs Per Colony Mating	922	42	803	117

1/ Generation 27 - Started July 9, 1984 through October 1, 1984
Generation 28 - Started March 25, 1985 through October 2, 1985

References Cited

Tanner, J. A., B. P. Weeks and M. Palmeri. 1983. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1982 - September 30, 1983:127-135.

Tanner, J. A., J. J. Baker and M. Palmeri. 1984. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1983 - September 30, 1984:77-79.

Project Number: GM 3.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: J. A. Tanner, J. G. R. Tardif, J. J. Baker and M. C. Flynn

This project concerns the development and evaluation of new rearing techniques and the improvement of presently used techniques. Unreliable or inefficient techniques will be modified or discarded.

The F_1 sterile male program is now the major user of reared insects. Currently, we can provide up to approximately 35,000 pupae/day. However, these numbers will have to be increased considerably in the future. To do this, the rearing facility will not only have to be enlarged, but new rearing techniques will have to be developed that will efficiently use all the available space.

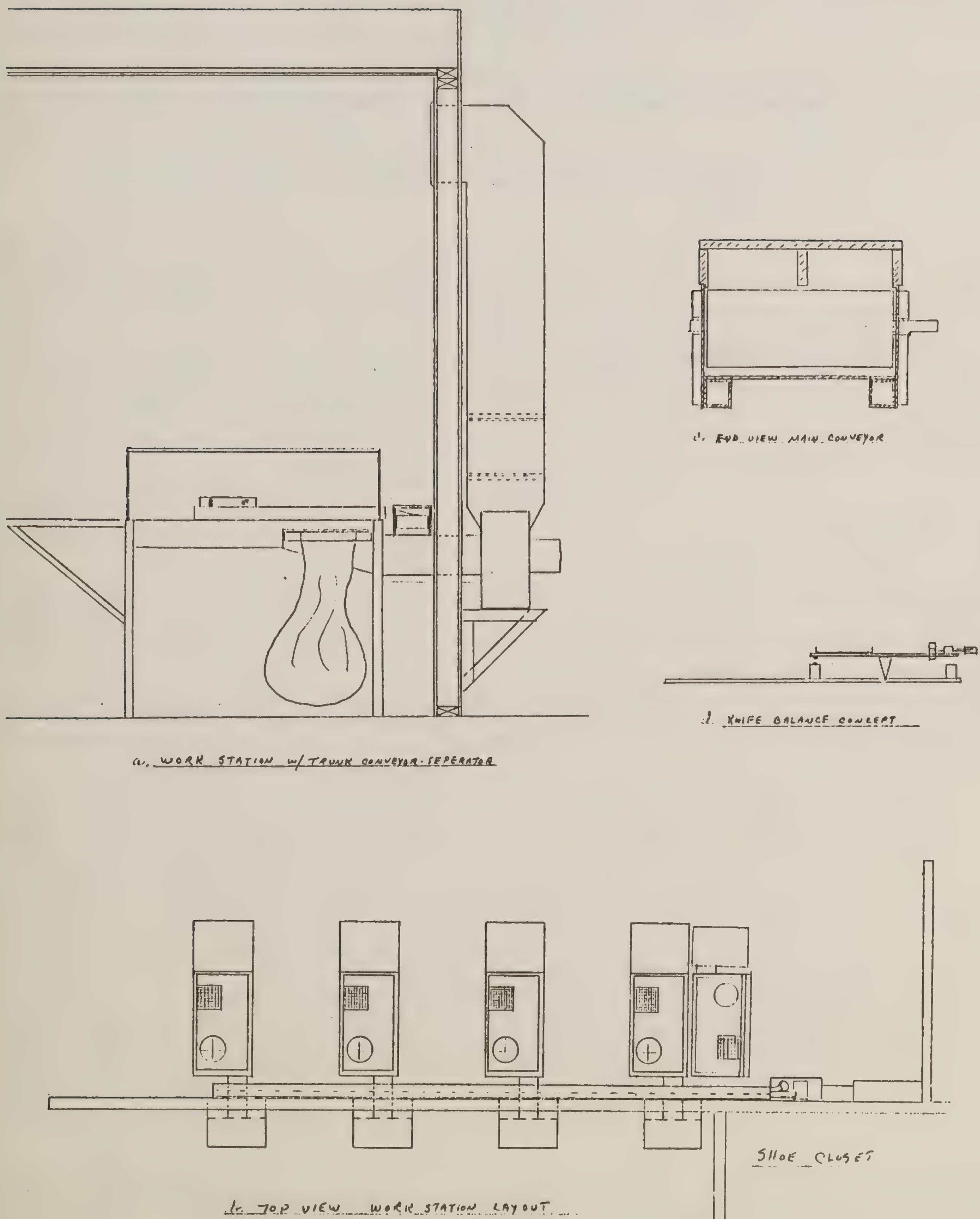
In the last report, we communicated that a conveyor system was constructed for moving newly havested pupae from the pupal harvesting tables to a common packaging area. The basic system is shown in Figure 1. A work station (Figure 1A) consists of a table with removable screened down-vent, a small 2-channel trunk conveyor (equipped with sexing hopper), a trash bag holding drawer, a plexiglass hood and an absolute filtered air evacuation and return system. This system prefilters the air twice before subjecting the air stream to an absolute filter.

Figure 1B depicts the arrangement of the four work stations in relation to the main 2-channel conveyor. A side view of the conveyor is shown in Figure 1C. The small trunk conveyors move the newly sexed pupae to the main conveyor. The main conveyor terminates into two separate weight counting knife balances. The balances are electromechanically coupled to trigger audio-visual indicators and control conveyor discharge. These balances allowed us to meter out 100 $\pm$ 10 pupae/container.

During the 1985 F_1 sterile male program, this system was used by 5 employees to harvest 35,000 pupae in a 6 hour period. In previous years, only about 2/3 that number could be harvested in the same amount of time.

Matings for the F_1 sterile male eggs are done in standard size grocery bags. Each bag holds 100 mating pairs and stand approximately 12" high after the top has been double folded to prevent the adults and/or their scales from escaping. An individual mating cart (5'6" high x 3'10" deep x 2'11" wide), can hold 3600 to 4800 mating pairs (36-48 bags). During the 1985 program, an average of 15,000 matings were done per day. This is equivalent to 3-4 carts/day. It was observed that most of the eggs were laid in the lower 3-4" of the bag, wasting 8-9" of bag space. An ideal mating container would be one that stands 3-4" high and made of rigid material that lends to mechanical harvest. Currently, the egg masses are scraped out of the bags into long rigid boxes (22" x 3" x 3") made of .040 plain chipboard. These boxes have a tight lid and might be suitable as a mating container. If 100 matings can be done per box, then as many as 16,800 matings could be done per cart.

Figure 1. An overall view of the new pupal harvesting table-conveyor system used during the 1985 F_1 sterile male program.



In late 1984, a small preliminary test was conducted with these boxes using normal New Jersey pupae. Table 1 shows that the number of eggs/mating was higher when 100 matings were done in the box, compared with the bag. However, increasing the number of matings/box to 200 resulted in a considerable reduction in the number of eggs/mating. Table 1 also shows that the percent hatch was the same for all treatments.

During the 1985 F_1 sterile male program, the boxes (100 matings) were tested against the bags. The number of eggs/mating and the percent embryonation are shown in Tables 2 and 3, respectively. Replicate 1 had a much lower number of eggs/mating than replicates 2 and 3. This seems to indicate that the insects were of poor quality. In this replicate, the boxes produced a lower number of eggs/mating and a lower percent embryonation.

In replicates 2 and 3, the number of eggs/mating was considerably higher when boxes were used for mating containers. The percent embryonation was similar for the two treatments.

The eggs are now in chilling to break diapause. The hatch results will be published in the next report.

Table 1. The effects of different mating-egg embryonation containers on the number of eggs deposited per mating by New Jersey F_{27} strain gypsy moth and the percent hatch of the eggs.

Type Container 1/	No. Matings/ Container	No. Eggs/ Mating	Percent Hatch
Bags (control)	100	714	93.9
Boxes	100	790	95.0
Boxes	200	614	95.3

1/ The gypsy moths were placed into the containers as pupae and eggs were harvested 35 days later.

2/ Bag matings were done in a standard grocery bag that was folded twice at the top. The box matings were done in a rigid box (22x3x3") made of .040 plain chipboard.

Table 2. The effects of different mating-egg embryonation containers on the number of eggs deposited per mating by gypsy moth females mated to partially sterilized males 1/ 2/

Type Container 3/	Number of Eggs/Mating 4/ Replicate 1	2	3	Mean 5/
Bags (Control)	500	672	655	609 ± 95
Boxes	473	731	712	639 ± 144

1/ Males exposed to 10 Krads as 8-11 day old pupae.
2/ Pupae were placed into the containers on the day the males were irradiated and the eggs were harvested 35 days later.
3/ Bag matings were done in a standard grocery bag that was folded twice at the top. Box matings were done in a rigid box (22x3x3") made of .040 plain chipboard.
4/ 100 matings/container.
5/ ± S.E.

Table 3. The effects of different mating-egg embryonation containers on the percent embryonation of eggs deposited by gypsy moth females mated to partially sterilized males. 1/ 2/

Type Container 3/ 4/	Percent Embryonation Replicate 1	2	3	Mean 5/
Bags (Control)	80	81	85	82.0 ± 2.6
Boxes	68	81	80	76.0 ± 7.0

1/ Males exposed to 10 Krads as 8-11 day old pupae.
2/ Pupae were placed into the containers on the day the males were irradiated and eggs harvested 35 days later.
3/ Bag matings were done in a standard grocery bag that was folded twice at the top. Box matings were done in a rigid box (22x3x3") made of .040 plain chipboard.
4/ 100 matings/container.
5/ ± S.E.

The F_1 eggs that are released into test plots near the time of wild egg hatch, must have a hatch profile which coincides with that of the wild. Eggs chilled 140-180 days at 7°C hatch coincidentally with wild eggs; F_1 eggs held longer than 180 days will hatch within the refrigerator.

Wild eggs begin hatching around March 15 in southern areas and around May 5 in northern areas. This means that all F_1 eggs must be produced over a 91-day period. In anticipation of higher production demands, we are looking for ways to widen this 91 day production "window"

We may be able to increase the production "window" by chilling the eggs at temperatures other than 7°C. We conducted tests to determine the hatch of New Jersey (strain used in F_1 program) eggs held under different chilling regimes. We also tried to determine if acclimating the eggs from 7 to 5 to 3°C would be of any benefit. Only the percent hatch has been analyzed at this time.

Figure 2 shows that holding the New Jersey eggs at temperatures lower than 7°C reduced the hatch; 3°C was detrimental to the eggs. At all three temperatures peak hatch occurred after 150 days. After 180 days of chilling, the eggs held at 7°C began to hatch. The percent hatch was 0 after 240 days of chilling because all the eggs had hatched within the refrigerator. No hatch occurred within the 3 and 5°C refrigerator.

Acclimating New Jersey eggs to 5°C by holding them at 7°C for 30 or 60 days enabled us to maintain a reasonable hatch (above 70%) at the 210 day chilling period (Figure 3). Acclimating the eggs not only eliminated the premature hatch, but also prevented the embryo mortality that occurs when eggs were held 210 days at 5°C. Eggs acclimated 60 days had a slightly lower percent hatch than eggs acclimated only 30 days. The percent hatch of the acclimated eggs dropped considerably after 210 days of chilling.

Eggs acclimated to 3°C by initially exposing them to various combinations of acclimation periods at 7°C and/or 5°C were benefited considerably when compared with eggs chilled continuously at 3°C (Figure 4). However, for the 210 day chilling period, all treatments had a lower percent hatch than eggs acclimated 30 days at 7°C followed by 180 days chilling at 5°C.

Another way to lengthen the production "window" may be to increase the embryonation period while keeping the chilling period 140 to 180 days. Present production protocol requires that F_1 eggs be embryonated 28-35 days (25°C). Under field conditions, wild eggs laid in early July may not be exposed to temperatures as low as 7°C until September or even October a 60 to 90 day period. If we could hold our eggs 60-90 days before chilling, we could increase our production period 30-60 days but still be able to give the eggs only 140-180 days of chilling.

We attempted to do this in the following experiment. Egg masses were held 28 (control), 56, 84, 112 and 140 days at 25°C and relative humidities of 50-55 (control), 55-60 or 80+% before being chilled at 3, 5 or 7°C. Each mass was sectioned into halves after 150 days of chilling. One half was dehaired and approximately 100 eggs were incubated at 25°C and 90% RH. The remaining halves were returned to chilling for an additional 30 days (180 days total chilling), then they too were dehaired and incubated.

At the present time, the percent hatch has been determined but not statistically analyzed. The hatch profile has yet to be evaluated.

Figures 5 and 6 show that high humidity is necessary if eggs are to be embryonated longer than 28 days and may be beneficial for eggs embryonated 28 days. The Figures only show the results for the 7°C chilling temperature, but the trend was similar for the 3 and 5°C temperatures. Only the high humidity results will be discussed. Chilling the eggs at 3 or 5°C was detrimental to the eggs (Figures 7 and 8). This agrees with the results of the previous experiment. In this experiment, we did not try to acclimate any of the eggs, but based on the results in the previous experiment, it should be evaluated.

The results evaluated thus far, seem to indicate that New Jersey eggs embryonated 56 days at 80+ humidity give the best hatch.

The data also seem to indicate that at the higher humidities, eggs could be held up to 112 days with only a minor loss in hatch, if the eggs are chilled 150 to 180 days at 7°C. If the hatch profile is acceptable, increasing the humidity and extending the embryonation period up to 112 days will enable us to open the production window by 84 days.

Figure 9 shows the percent hatch of the F_1 partially sterilized eggs produced during the 1984 program. These eggs were embryonated at a 50-55% RH for 28 days and chilled at 7°C. The Figure shows that the optimum length of chilling is 150 days. Longer periods of chilling resulted in premature hatch.

Figure 2. The percent hatch of New Jersey F_{27} eggs chilled for 120 to 240 days at 3, 5, or $7^{o}C$.

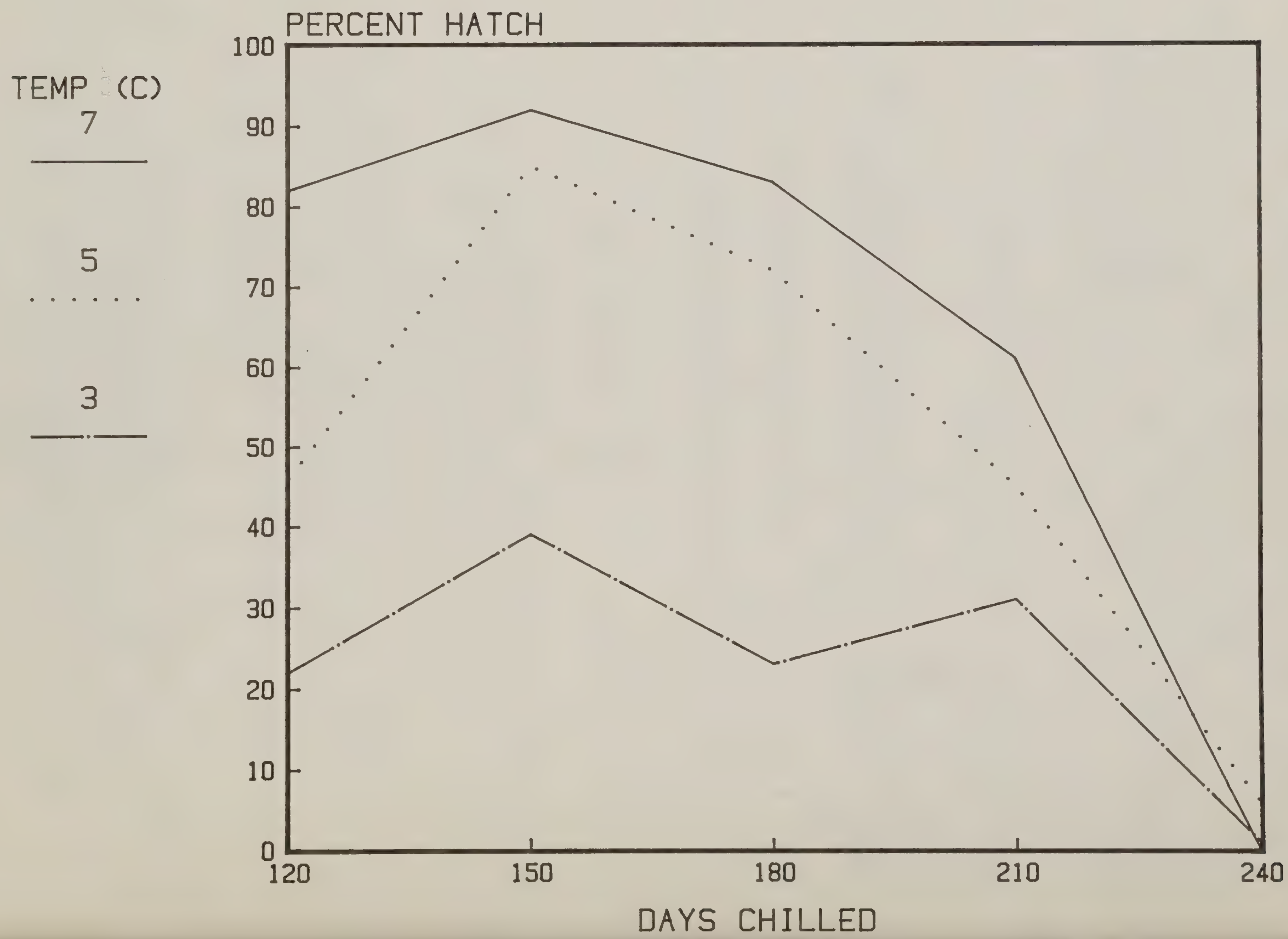


Figure 3. The percent hatch of New Jersey F_{27} eggs chilled at $5^{o}C$ after a 30 or 60 day acclimation period ($7^{o}C$).

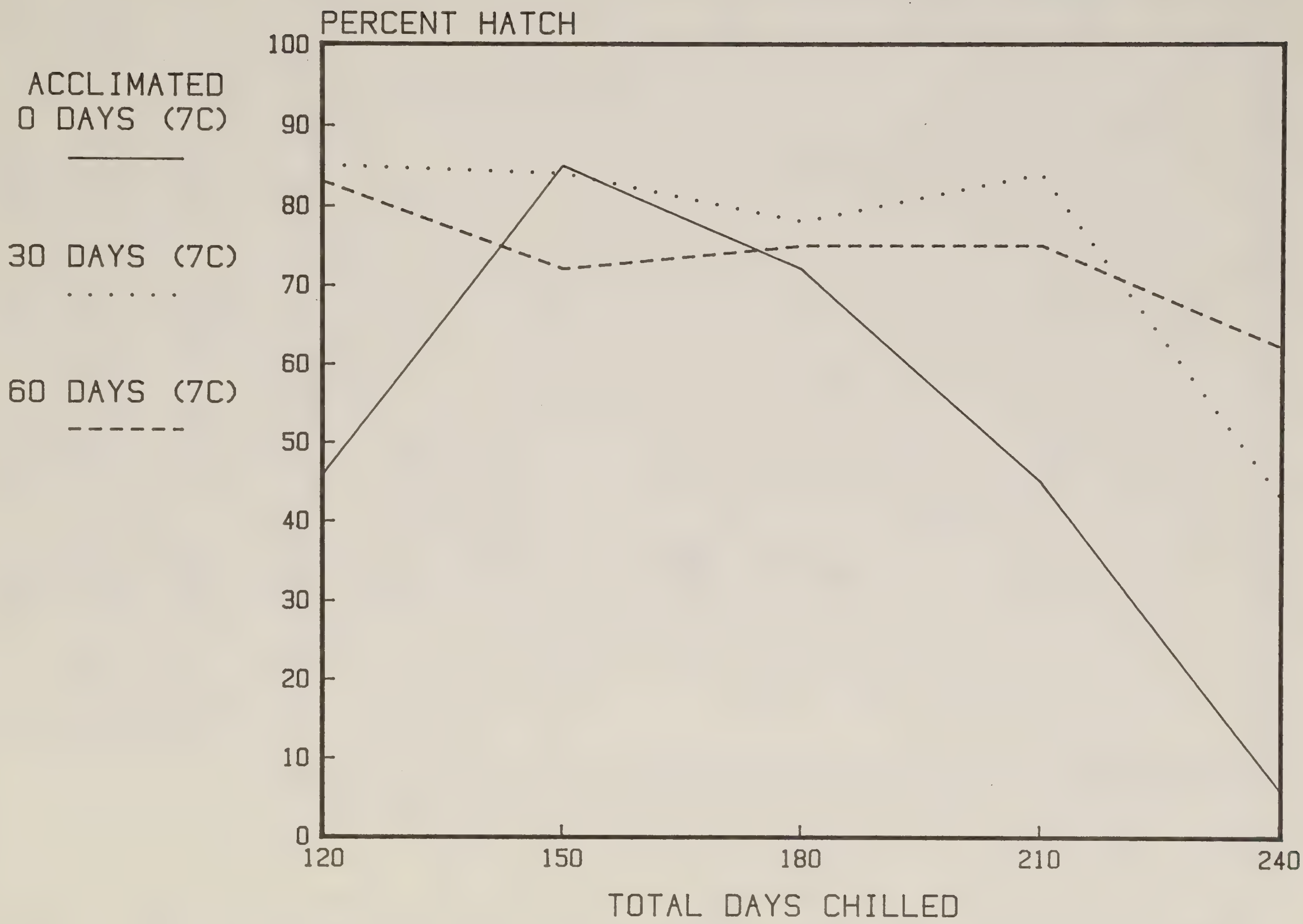


Figure 4. The percent hatch of New Jersey F_{27} eggs chilled at $3^{o}C$ after various combinations of acclimation periods at $7^{o}C$ and/or $5^{o}C$.

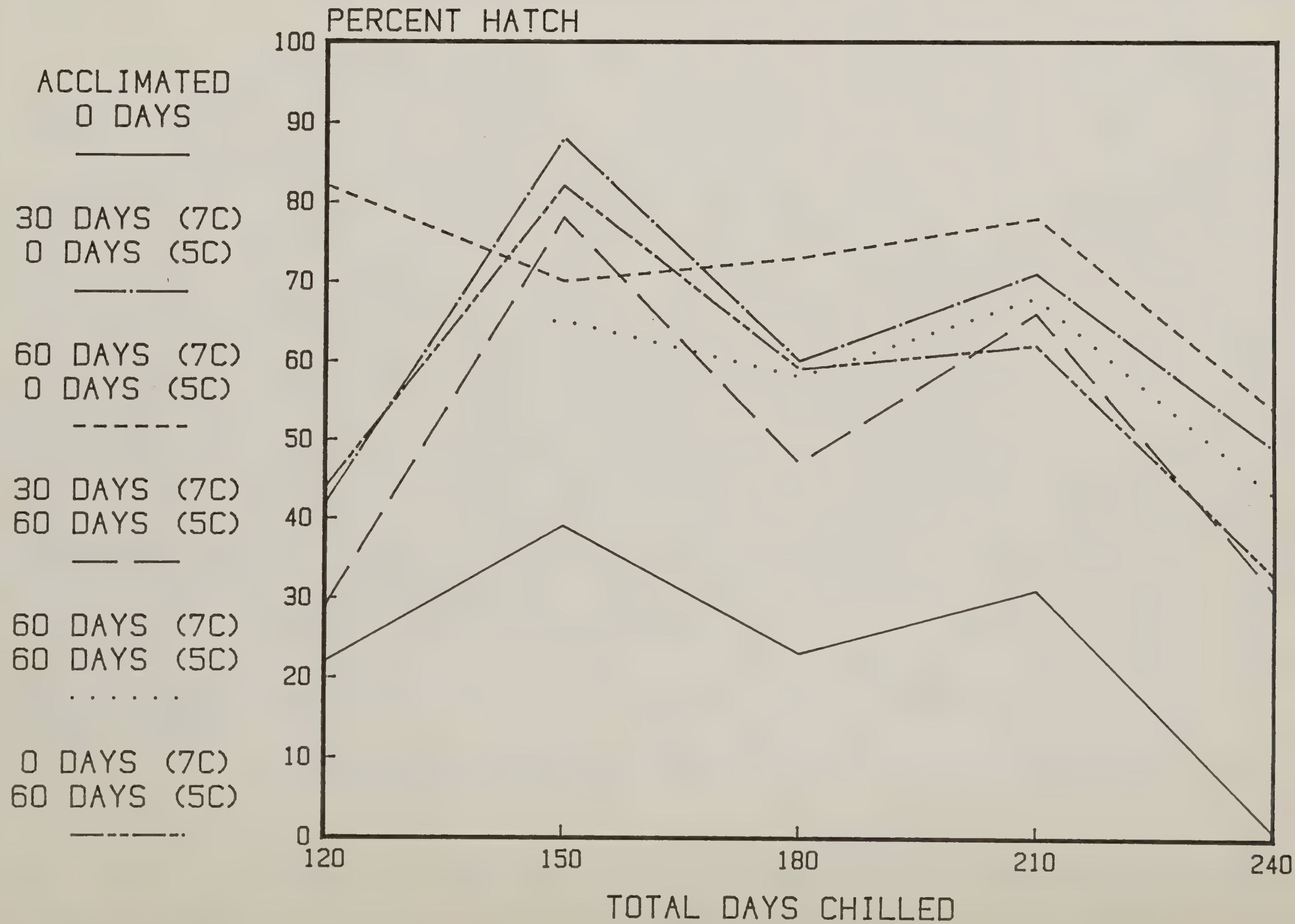


Figure 5. The percent hatch of New Jersey F_{27} eggs embryonated 28 to 140 days at relative humidities of 50-55, 55-60 or 80+% and chilled 150 days at $7^{\circ}C$.

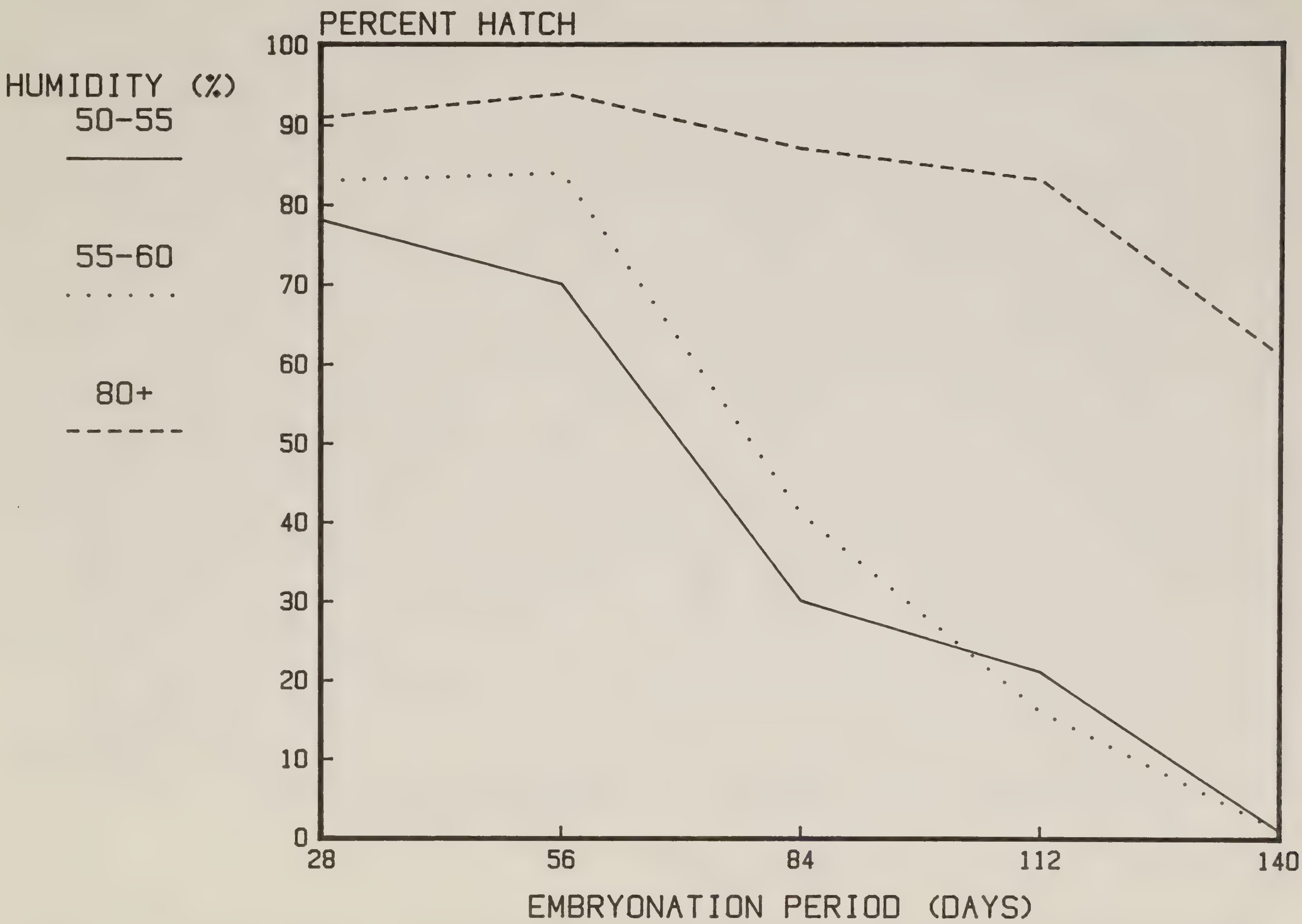


Figure 6. The percent hatch of New Jersey F_{27} eggs embryonated 28 to 140 days at relative humidities of 50-55, 55-60 or 80+% and chilled 180 days at $7^{o}C$.

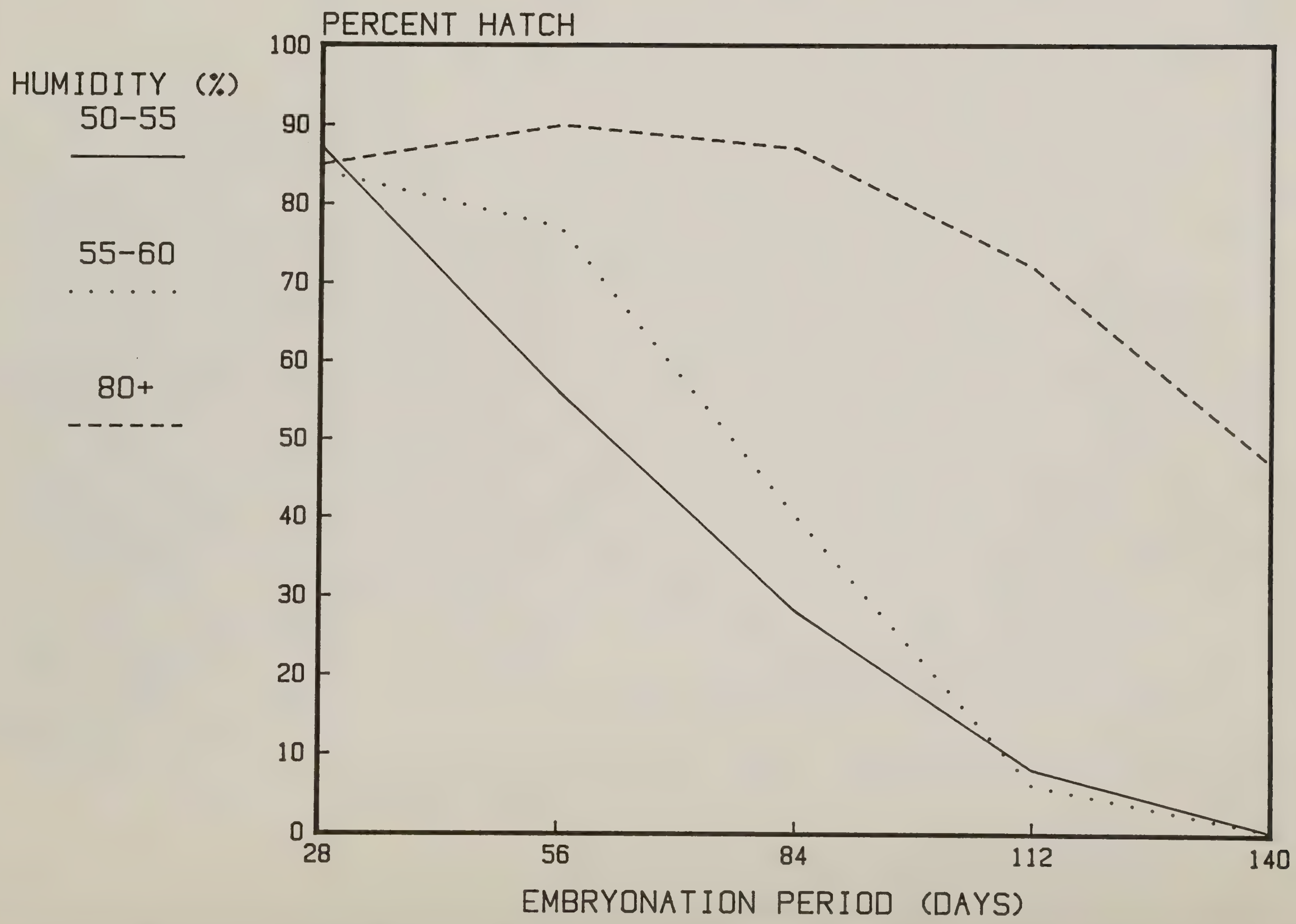


Figure 7. The percent hatch of New Jersey F_{27} eggs embryonated 28 to 140 days at 80+% humidity and chilled 150 days at 3, 5 or 7°C.

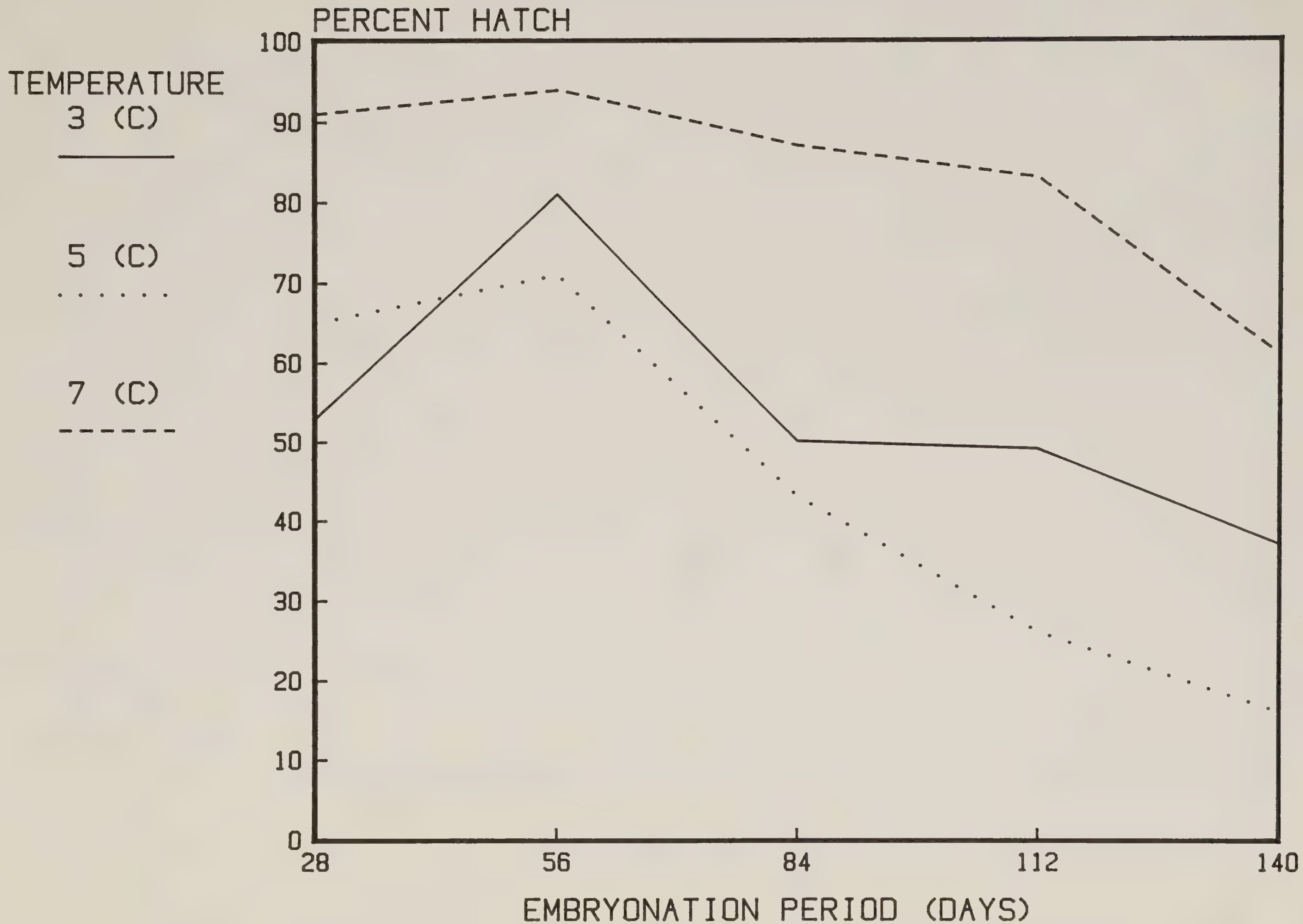


Figure 8. The percent hatch of New Jersey F_{27} eggs embryonated at 80+% humidity for 28 to 140 days and chilled 180 days at 3, 5 or $7^{\circ}C$.

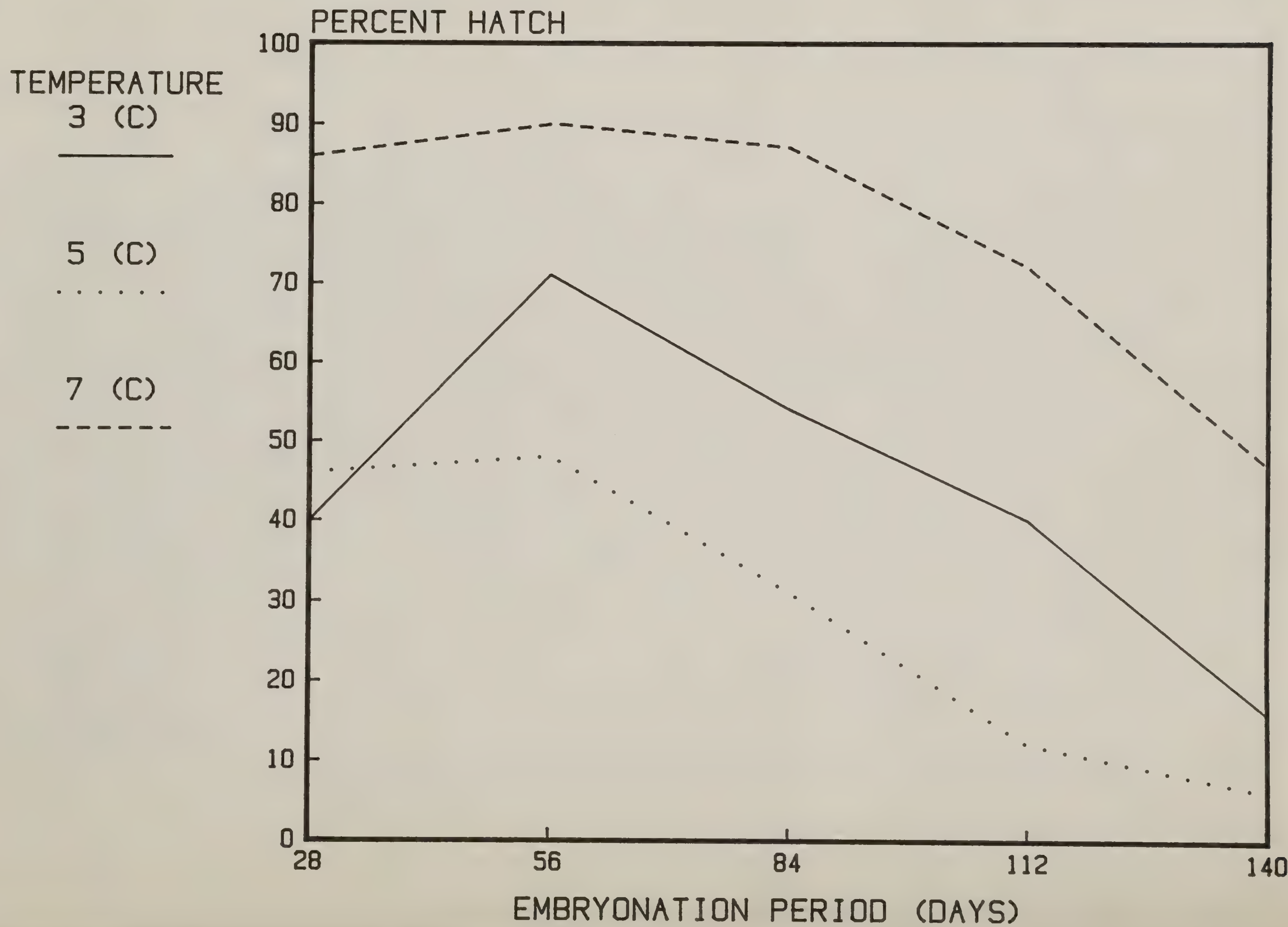
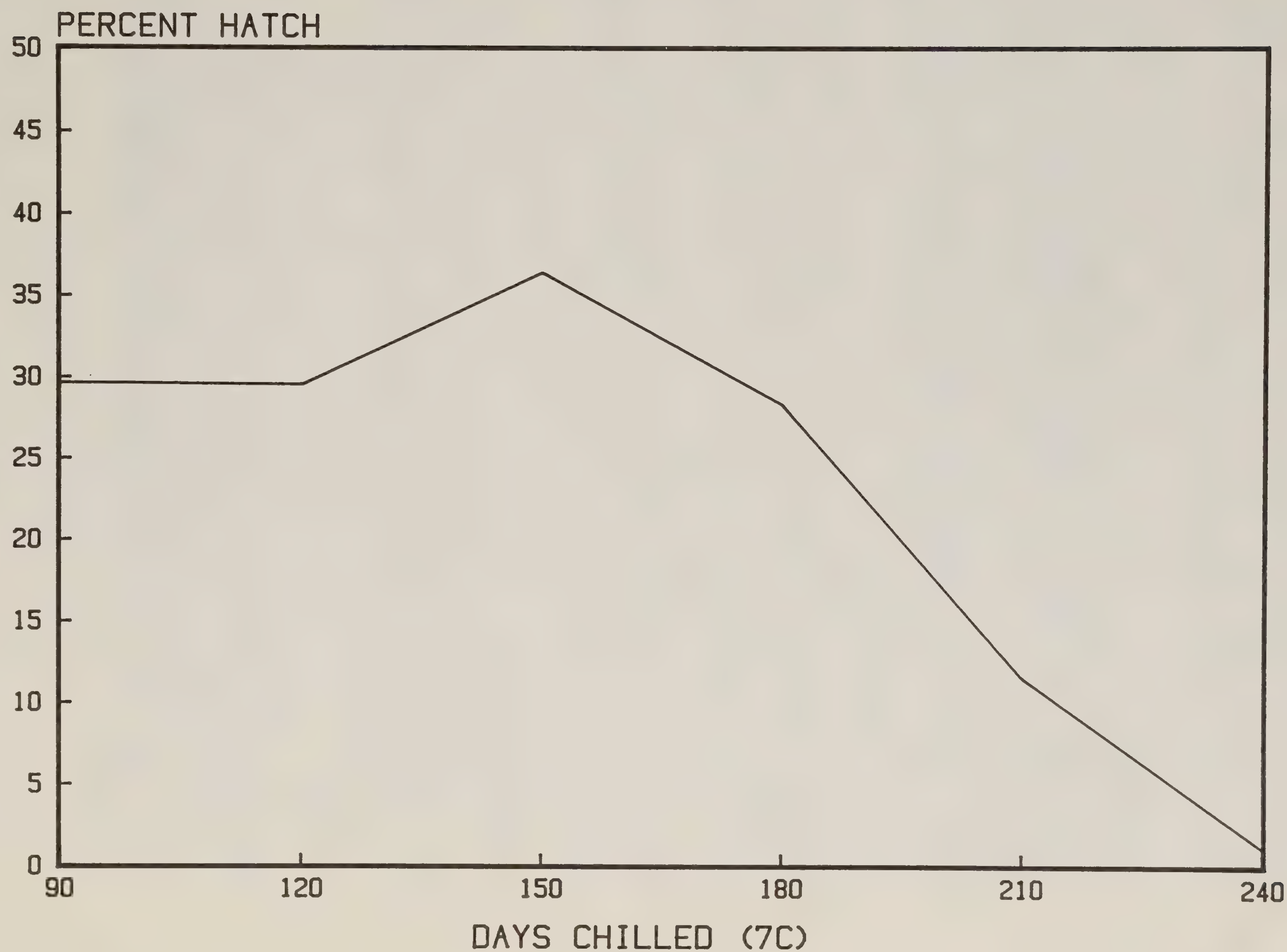


Figure 9. The percent hatch of F_1 partially sterilized eggs produced in the fall of 1984.



Project Number: GM 4.3.1
Project Title: The Number of Instars in the New Jersey Standard Strain
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: J.A. Tanner, J.J. Baker, and M.C. Flynn

Introduction:

In the F_1 sterile male program, male and female pupae are routinely harvested four days after 10% male pupation has occurred (Tanner and Weeks, 1981). Early in the 1984 rearing program, it was noticed that more female larvae (therefore, fewer pupae) were present at the time of pupal harvest than had been observed in the 1983 program. This resulted in a considerable reduction in the number of F_1 matings (since few females were available) and quickly forced the abandonment of the "10% male pupation plus four days" harvest trigger. In order to meet the production schedule, a time consuming "double harvest" techinque was implemented (each container was harvested twice, at 7 day intervals).

The New Jersey laboratory strain used in the F_1 sterile male program was previously observed to produce few six-instar type female larvae (Bell, personal communication). However, in tests conducted last year, almost 33% of the female larvae were of the six and seven instar type (Tanner, et al, 1984). Their developmental time to pupation was 8 days longer than the male larvae and the five instar type female larvae.

Prior to the 1985 F_1 sterile male program, the colony was sampled to determine if there still was a high percentage of six and seven instar type female larvae in the population. This was done to determine if the "double harvest" technique would have to be used this summer.

Methods and Materials:

Surface disinfected and dehaired New Jersey F_{27} eggs were placed into 30 6-oz. fluted cups containing 85 ml. of B-4 diet. Newly hatched neonates were transferred into individual 1-1/2 oz. Thunderbird cups containing 24 ml of B-4 diet. Each cup was checked daily and after each molt, the head capsule and cast skin were removed.

Results:

Table 1 shows the percentage of male and female larvae pupating after the 4th, 5th, 6th and 7th instar in 1984 and 1985. There was definitely a shift towards fewer instars in both sexes in 1985. In 1985, the males had a similar percentage of 5th instar type larvae as in 1984. However, they also had almost an 11% increase in 4th instar type larvae and a 19% reduction in 6th instar type larvae. In 1984, over 2% of the male larvae and almost 5% of the female larvae were of the 7th instar type. In 1985, there were no 7th instar type larvae.

Table 2 shows that, based on instar type, the time to pupation was shorter in both male and female larvae in 1985 than in 1984. What caused the acceleration in larval development and the shift towards fewer instars in 1985 is not known at this time. However, during the 1984 study, there was a problem with larval "straggling" (see Tanner, et al, 1984). Larval "straggling" or "stunting" is characterized by the lack of or reduction in growth in some newly hatched larvae. Electron microscopic examination of stunted larvae has shown that these larvae contain a high concentration of a Rickettsia-like organism (RLO) which, when fed to cabbage looper larvae, caused stunting in that species (ODell, personal communication). Normally developing larvae were also found to contain RLO, but in lower concentration. It is possible that the concentration of RLO may affect the speed at which the larvae develop and/or the number of instars.

Table 1. The percentage of male and female larvae pupating after the 4th, 5th, 6th and 7th instar in 1984 and 1985.

		Number of Instars Prior to Pupation			
Year	Sex	4	5	6	7
1984	Male	3.7	88.0	5.8	2.4
	Female	0.3	67.0	28.1	4.6
1985	Male	14.6	84.8	0.6	0
	Female	0	90.8	9.2	0

Table 2. The number of days to pupation of 4th, 5th, 6th and 7th instar type male and female larvae in 1984 and 1985.

		Number of Instars Prior to Pupation 1/			
Year	Sex	4	5	6	7
1984	Male	27.4 ± 1.1	31.0 ± 3.0	44.1 ± 10.1	54.0 ± 7.4
	Female	32.0 ± 0.0 2/	31.9 ± 2.0	39.3 ± 4.2	55.1 ± 8.4
1985	Male	25.9 ± 1.6	27.0 ± 2.2	46.0 ± 7.1 3/	-----
	Female	-----	29.9 ± 2.0	34.1 ± 3.0	-----

1/ ± S.E.
2/ Only one female larvae was of the fourth instar type.
3/ Only two male larvae were of the sixth instar type.

References Cited

Tanner, J.A., J. A. Baker and M. Palmeri. 1984. Evaluating the development and reproduction of insects produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1983 - September 30, 1984:77-79.

Project Number: GM 4.3.2
Project Title: Development of Strontium as a Marker for Gypsy Moth Larvae
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: J.A. Tanner, L.L. Herbough, D. Burns, V. Douville and R. Demanche

Introduction

The gypsy moth sterile male program involves the release of F-1 eggs which develop into sterile adults. These eggs are produced by mating normal females with males exposed to substerilizing dosages of radiation in the pupal stage (Lance, et al, 1983). A critical need for this program is a method of marking the F-1 insects so they can be monitored after release.

Metals such as strontium, rubidium and cesium have been successfully used as markers in adult Lepidoptera and Diptera by incorporating the metal directly into the larval diet (Van Steenwyk et al, 1978, Moss and Van Steenwyk 1982, 1984, Burns et al 1983, Legg and Chiang 1984). The purpose of this project is to determine if strontium is maternally transmitted to F-1 eggs and how long it can be detected in F-1 larvae.

Results and Conclusions

The last annual report described the methods and materials and gave a summary of the results. Initially, the offspring of gypsy moth larvae reared on diet containing strontium ($SRCL_2$) was found to have a higher concentration of strontium in their tissue than control larvae. However, within 4 days of feeding on normal diet, the level of strontium in their tissue decreased to a level similar to that of the control larvae. This rapid elimination of strontium from the tissue of the F-1 larvae made it impractical to use stronium as a marker of late instar F-1 sterile larvae by feeding it to the parents.

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Legg, D.E. and H.C. Chiang. 1984. Rubidium Marking Technique for the European Corn Borer (Lepidoptera:Pyralidae) in Corn. Environ. Entomol. 13:579-583.

Moss, J.I. and R.A. Van Steenwyk. 1982. Marking Pink Bollworm (Lepidoptera: Gelechiidae) With Cesium. Environ. Entomol. 11:1264-1268.

Moss, J.I. and R.A. Van Steenwyk. 1984. Marking Cabbage Looper (Lepidoptera: Noctuidae) With Cesium. Environ. Entom. 13:390-393.

Van Steenwyk, R.A., G.R. Ballmer, A.L. Page and H.T. Reynolds. 1978. Marking Pink Bollworm with Rubidium. Ann. Entomol. Soc. Am. 71:81-84.

Project Number: CNPPSDP 4.1.1
Project Title: Pheromone-Based Survey Technology for Early Detection of Exotic Insect Pests
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: V. C. Mastro, C. P. Schwalbe and P. C. Kingsley
Project Cooperators:

M. Kirby, International Programs, USDA, APHIS
Canberra, Australia
S. Wilson, International Programs, USDA, APHIS
New Delhi, India
Drs. M. Vaissayre, B. Sauphanor and N. Doualehi
Ministere de L'Education National
Et de La Recherche Scientifique
Institut des Savanes, Centre Textile
BP 604, Bouake', Ivory Coast, Africa
Dr. P. Newton, Citrus and Subtropical Fruit Research Inst.
Nelspruit, South Africa
R. T. Staten, Pink Bollworm Methods Development Laboratory
USDA, APHIS Phoenix, Arizona
Dr. D. R. Hall, Tropical Development and Research Institute
56-62 Gray's Inn Road, London, England
Jerome Jones, Florida A & M University

The objective of this program is to provide pheromone-based survey technology for detection of introductions of exotic pests. The following report summerizes all tests, both domestic and foreign, relating to this objective including trap design, bait evaluation and pheromone combination tests. It also includes results of the 1985 pilot scale survey for six exotic species. The current "1986" survey recommendations for thirteen exotic pests is included.

Domestic Studies

Gypsy moth *Lymantria dispar*

Pheromone baits for various exotic species were tested for compatibility in gypsy moth traps in 1985. Although several good combinations have already been identified, (ie. earlier studies have tested the compatibility of baits for fourteen species: 1984 Annual Report CNPPSDP 4.1.1) identification of additional combinations would allow more flexibility and better utilization of resources in the large gypsy moth trapping program. Table 1 presents the results of the 1985 bait combination trials on gypsy moth. For testing, traps were placed in a natural gypsy moth infestation on lines with 50 m between traps and between lines (replicates) spacing. Traps were suspended from metal stakes at ca. 1 meter in height to negate the effect of tree size boles. Traps were checked and randomized daily for the duration of the test.

Two bait combinations did not result in trap captures significantly lower than the control; GM + CS and GM + AG (Table 1). Traps baited with the remaining five combinations captured significantly fewer numbers of gypsy moths and would not be suitable for combination trapping.

Table 1. Mean numbers of gypsy moth, Lymantria dispar, males captured in traps[1/] baited with L. dispar pheromone alone and in paired combination with a variety of other attractants. Test was performed near Otis ANGB, MA 7/24 - 8/1, 1985.

Treatment 1	Combination 2	$\bar{x}$ Number of Males Per Trap Per Reading
GM her-lam	Control	102.0 a
GM her-lam	CS ray-rs	91.1 a
GM her-lam	AG ray-rs	87.2 a
GM her-lam	CP ray-rs	71.9 b
GM her-lam	MB lab-pc	36.24 c
GM her-lam	CL tre-rs	18.42 d
GM her-lam	PS alb-fib	21.67 d
GM her-lam	EI zoe-pc	8.80 e

1/ USDA milk carton traps used for all treatments
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Five complete replicates were read and randomized nine times for a total of fourty-five observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Trap design tests were also conducted with the gypsy moth during the 1985 field season. The purpose of these trials was to determine if alternative trap designs can be used in gypsy moth trapping programs when combination trapping for an exotic pest is being planned. The standard traps (delta and milk carton) now used for gypsy moth programs, are not efficient for capturing several of the exotic pests which could be trapped in combination programs.

In the first test, the standard USDA-delta trap was compared to the USDA milk carton trap and four commercially produced traps. For testing, traps were placed on lines with a 50 m inter-trap and inter-line spacing. Traps were checked and cleared several times a day. Frequent checking and clearing of traps was necessary to prevent loading of traps that depend on a sticky surface for moth capture (ie. delta, INRA-delta-w.o. and Inter-delta).

All of the four commercial trap designs tested compared favorably with the standard USDA delta and milk carton traps (Table 2). Three of the designs caught significantly more males than the standard delta traps. It appears that if any of these four designs are equally efficient in capturing the candidate exotic target species in planned combination programs, that they will not compromise the efficiency of a gypsy moth trapping survey.

In a second test of trap designs, the efficiency of the delta trap was compared with the trap now used in the exotic pest detection program (Pherocon 1C) and also with the large delta trap (Inter-delta) tested in Trial one. Traps were placed on lines 50 m apart with a 50 m inter-trap spacing. Five complete replicates (lines) were established in an area with a native population of gypsy moths. To prevent overloading, traps were checked and cleared of moths every two hours and at the same time, the traps on a line were rerandomized. In all, the traps were read and randomized ten times (8/12 - 4 times, 8/14 - 3 times and 8/21 - 3 times).

Results of this trap design test are presented on Table 3. Similar to the first test, the Inter-delta captured significantly more males than the standard USDA-delta. Also, the P-1C trap performance was not significantly different from the USDA trap, indicating that either of these traps can be used in programs where combination trapping for the gypsy moth is planned.

Table 2. Mean number of gypsy moth *Lymantria dispar* males captured in traps1/ of various designs baited with gypsy moth pheromone dispensers.2/ Test was performed in Yarmouth, MA 7/19/85-8/2/853/

Trap Design	$\bar{x}$ Number of Gypsy Moths Captured per Trap3/
Milk Carton	5.2 b c
INRA-delta-w.o.	7.2 a b
Multi-2	8.8 a b
Inter-delta	9.8 a
Multi-1	6.2 c
or delta-ec	4.5 c

1/ Trap types, see Appendix 4.
2/ Standard 1985 gypsy moth lure dispensers (lot D0175 - Hercon laminate) were used to bait all traps.
3/ Six complete replicates were read and randomized eight times.
4/ Means followed by the same letter are not significantly different at the 5% level accoring to Duncans Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Table 3. Mean number of gypsy moth *Lymantria dispar* males captured in traps[1/] of various designs baited with gypsy moth pheromone dispensers.[2/] Test was conducted near Otis ANGB 8/12, 8/14 and 8/21 1985.[3/]

Trap Design	$\bar{x}$ Number of Gypsy Moths Captured per Trap[4/]
Inter-delta	104.2 a
P-1C	74.0 a b
or delta-ec	56.0 b

1/ Trap types, see Appendix 4.
2/ Standard 1985 gypsy moth lure dispensers (lot D0175 - hercon laminate) were used to bait all traps.
3/ Five complete replicates were read and randomized ten times.
4/ Means followed by the same letter are not significantly different at the 5% level accoring to Duncans Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Australian Studies

Light brown apple moth, *Epiphyas postvittana*

The objective of these trials was to determine if combination trapping is feasible with one of the target species now included in our operational exotic pest survey; the light brown apple moth (LBAM). In apple orchards, lines of traps were established with 40 m between line and between trap spacing. Traps were suspended from the limbs of apple trees at approximately one meter in height. Five complete replicates of this test were established and checked and randomized four times.

Results of this trial demonstrate that pheromones for several species, when placed in traps baited for LBAM do not significantly reduce trap catch (Table 4). These combinations will be considered in trapping programs if LBAM pheromone does not inhibit capture of the other candidate target species. Of the two trap designs tested, the USDA delta trap (or-delta-ec) captured significantly fewer males than P-1C traps similarly baited. Several other trap designs are presently being tested to determine if more efficient designs and/or designs which do not rely on sticky surfaces can be developed.

Table 4. Mean numbers of light brown apple moth, *Epiphyas postvittana*, males captured in traps baited with *E. postvittana* pheromone dispensers alone and in paired combination with various other attractants. Test was performed in Huon Valley, Tasmania, Australia, March 18 - 22, 1985.1/

Attractant Combination2/ 1	2	Trap Type3/	$\bar{x}$ No. of Males4/ Captured/Trap/Day
LBAM lab-rs	CONTROL	P1C	4.15 a
LBAM lab-rs	--------	or-delta-ec	1.65 b
LBAM lab-rs	GM her-lam	P1C	5.20 a
LBAM lab-rs	CM alf-fib	P1C	4.75 a
LBAM lab-rs	PFM alb-rs	P1C	5.55 a
LBAM lab-rs	ADOX alb-pc	P1C	0.05 c
LBAM lab-rs	FCM alb-fib	P1C	3.85 a
LBAM lab-rs	LB alb-rs	P1C	3.45 a
LBAM lab-rs	EA alb-rs	P1C	2.85 a
LBAM lab-rs	AV alb-fib	P1C	0.05 c
LBAM lab-rs	GP alb-rs	P1C	4.60 a
LBAM lab-rs	PV alb-fib	P1C	1.00 b
LBAM lab-rs	OFM alb-fib	P1C	3.19 a
LBAM lab-rs	TAB zoe-pc	P1C	0.55 b c

1/ Five complete replicates were read and randomized four times = twenty observations per treatment.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Trap type codes in Appendix 4.
4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

India Studies

Spiny bollworm, *Earias insulana*

Studies were conducted in India to test various trap designs for efficiency in capturing the spiny bollworm, *Earias insulana* (EI), and to determine which pheromone lures for various species could be placed in EI baited traps without inhibiting EI captures. Five lines of traps (replicates) were established in a cotton field with a 30 m between line and 25 m between trap spacing. Traps were placed on stakes so that the trap was approximately at crop height. Traps were read and rerandomized three times.

Results of this test are presented in Table 5. Of the four trap types tested, the P-1C trap captured the greatest number of males and this was significantly greater than either of the two configurations of the delta trap tested. Pheromone baits for nine species, when placed in traps baited for EI, did not significantly reduce the number of males captured and are potential candidates for combination trapping. Also presented in Table 5, are the numbers of *Pectinophora gossypiella* (PBW) and *Spodoptera litura* (CL) males captured in traps. As expected, traps baited for these species captured large numbers of the respective species. In the case of *S. litura*, large numbers of males were also captured in traps baited for ECL, *Spodoptera littoralis*. The same two compounds, in slightly different ratios, are used for baiting for both species and cross attraction is known to occur. Both pheromones also appear to be strong inhibitors for EI males. However, the numbers of CL captured were so large that they simply might have loaded the traps and physically excluded EI males. Investigation would be necessary to determine if inhibition or exclusion is occurring.

Interestingly, large numbers of PBW were captured in traps baited with a combination of EI + FCM (*Cryptophlebia leucotreta*) pheromone dispensers. An earlier trial, testing various combinations of lures for attractancy to PBW demonstrated that FCM lure significantly reduced PBW captures (1984 Annual Report CNPPSDP 4.1.1, Table 6, p. 114).

Table 5. Mean numbers of spiny bollworm, *Earias insulana* males, pink bollworm, *Pectinophora gossypiella* males and rice cutworm, *Spodoptera litura* males captured in traps baited with *E. insulana* pheromone alone and in paired combination with attractants for various other species. Test was conducted in Hissar, India 8/21/85 - 9/15/85.1/

Attractant Combination2/ 1	2	Trap type3/	$\bar{x}$ No. of *E. insulana* captured per trap4/	$\bar{x}$ No. of *P. gossypiella* captured per trap	$\bar{x}$ No. of *S. litura* captured per trap
EI tre-pc	HZ alb-fib	P-1C	45.6 a	0.2	0.0
EI tre-pc	CP ray-rs	P-1C	40.2 ab	0.4	0.0
EI tre-pc	HA alb-fib	P-1C	32.4 ab	0.8	0.2
EI tre-pc	CS ray-rs	P-1C	28.8 abc	1.0	0.0
EI tre-pc	CONTROL	P-1C	29.2 abcd	1.0	0.2
EI tre-pc	GM her-lam	P-1C	14.8 bcd	2.2	0.2
EI tre-pc	HV alb-fib	P-1C	9.4 cde	7.8	0.0
EI tre-pc	AG ray-rs	P-1C	11.4 cde	1.6	0.0
EI tre-pc	FCM alb-fib	P-1C	7.8 cde	77.0	0.0
EI tre-pc	-----------	INRA delta	9.0 de	0.2	0.0
EI tre-pc	PBW alb-fib	P-1C	7.0 de	124.0	0.0
EI tre-pc	PS alb-fib	P-1C	5.8 ef	1.8	0.2
EI tre-pc	HP alb-fib	P-1C	4.6 ef	0.6	0.0
EI tre-pc	NA zoe-rs	P-1C	5.0 ef	0.4	0.2
EI tre-pc	-----------	or delta eo	5.2 efg	0.0	0.0
EI tre-pc	MB lab-pc	P-1C	1.4 fgh	1.6	0.0
EI tre-pc	ECL ray-rs	P-1C	1.0 gh	0.6	21.4
EI Blank	-----------	P-1C	0.6 gh	1.6	0.0
EI tre-pc	-----------	or delta ec	0.4 h	0.2	0.0
EI tre-pc	CL tre-rs	P-1C	0.0 h	0.0	57.8

1/ Five complete replicates were read and randomized three time = fifteen observations per treatment.

2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.

3/ Trap type codes in Appendix 4.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Studies in Africa were conducted in cooperation with the Ivory Coast Ministere de L'education Nationale et de la Recherche Scientifique at the Institut de Savannes, Centre Textile. The office of International Cooperation and Development (OICD) sponsored this project. Trials were conducted at or near the IDESSA Textile Research Station in Bouake'. Trap and pheromone trials were conducted on five species: *Spodoptera littoralis*, *S. exempta*, *Cryptophlebia leucotreta*, *Pectinophora gossypiella* and *Heliothis armigera*. We attempted to conduct studies with three additional species: *Earias insulana*, *E. biplaga* and *Diparopsis watersi*, however, during the period of our stay, populations of these species were too small for meaningful field tests.

Information and specimens were also gathered for a number of other species including *Sylepta derogata*, a pest of cotton, *Eldana saccharina*, a pest of sugarcane and corn, and *Nymphula depunctalis*, a pest of rice.

Egyptian cotton leafworm, *Spodoptera littoralis*

In a preliminary study designed to determine if any of four commercial formulations of pheromone for *S. littoralis* (ECL) were superior, they were tested in a small field trial. For testing, traps baited with the various formulations, were placed in cotton fields on lines (30 m spacing) and with a 30 m inter-trap spacing. All traps were hung from wooden stakes so that the traps were slightly above the crop height. Lines of traps (4 replicates) were checked and randomized twice. A fifth treatment was added to this test to determine if pheromone for the red bollworm *Diparopsis watersi* (RB) had any synergistic effects on traps baited for *S. littoralis*. Studies in South Africa (see 1984 Annual Report, Table 10, p. 120) indicate that the combination of these two lures significantly increased captures of ECL males.

Results of this test are presented in Table 6. Of the four commercial formulations tested, the largest numbers of males were captured in traps baited with the ECL pheromone formulation distributed by TRECE (ECL tre-pc). However, analysis could not detect significant differences between the four formulations. Additions of RB lure to traps baited for ECL, in this trial, did not appear to enhance captures of ECL males.

Table 6. Mean numbers of Egyptian cotton leafworm <u>Spodoptera littoralis</u> males captured in traps 1/ baited with various commercial formulations of <u>S. littoralis</u> pheromone and with one commercial formulation tested in combination with <u>D. castanea</u> pheromone. Test performed in Bouake', Ivory Coast, Africa September 25-27, 1985.

Treatment 2/	$\bar{x}$ Number of Males Captured Per Trap for 2 Readings 3/
ECL her-lam	15.3 a 4/
ECL ray-rs	6.8 a
ECL tre-pc	23.5 a
ECL zoe-rs	7.3 a
ECL zoe-rs + RB lab-pc	5.0 a

1/ Wing type Pherocon-1C traps used for all treatments

2/ Species codes, manufacturer codes and dispenser types in Appendis 1, 2 and 3, respectively

3/ Four complete replicates were read and randomized twice for eight observations per treatment

4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

The following three trials were conducted to determine which pheromones of various other species can be combined with ECL lures without inhibition of ECL trap captures. All three trials were conducted in cotton fields. Trap spacing (30 m) and line spacing (30 m) was also the same for all three trials. Similar to the previous formulation test, all traps were suspended from stakes so that the trap was slightly above the crop height. Also, in all three trials, treatments were replicated four times and traps were read and randomized three times.

Results of the first of the ECL lure combination tests is presented in Table 7. Of the four combinations of lures tested, traps baited with all combinations captured fewer numbers of males (not significantly different) than the control. Interestingly, traps baited with the combination of ECL and RB (red bollworm) lures in this trial, resulted in the largest number of males captured, but this was not significantly greater than traps baited with ECL pheromone alone. Another exotic pest, the false codling moth (FCM), was known to be present in the cotton fields where this test was conducted. Therefore, traps baited as FCM controls were included in this trial.

Table 7. Mean number of Egyptian cotton leafworm, Spodoptera littoralis, males and false codling moth, Cryptophlebia leucotreta, males captured in traps 1/ baited with their respective pheromones alone and in traps baited with ECL pheromone in combination with various other attractants. Test was performed in Bouaké, Ivory Coast, Africa September 27 - October 1, 1985.

Treatment (Attractant Combination) 2/ 1	2	$\bar{x}$ No. of ECL males captured/trap for 3 readings 3/	$\bar{x}$ No. of FCM males captured/trap for 3 readings
ECL tre-pc	CONTROL	37.5 a 4/	0.0 4/
ECL tre-pc	PBW USDA-rs	47.3 a	0.0
ECL tre-pc	PS alb-fib	44.8 a	0.0
ECL tre-pc	RB USDA-pc	61.8 a	0.0
ECL tre-pc	FCM tre-rs	36.3 a	24.8 a
CONTROL	FCM tre-rs	0.0	117.0 b

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Four complete replicates were read and randomized three times for a total of twelve observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Although the addition of FCM lures to ECL traps did not inhibit ECL captures, this combination did significantly reduce captures of FCM males.

Results of the second of the ECL combination trials are presented in Table 8. Of the five lure combinations tested, only one resulted in significantly lower captures than the control (ECL tre-pc + NA tre-rs). The other four combinations, in terms of impact on ECL captures, were not significantly different from the control and should be candidates for combination trapping. Again, because it was known that one of the species, Spodoptera exempta (NA), was present in the cotton fields in which the test was conducted, traps baited as NA controls were included. These NA controls captured significantly more NA males than traps baited with a combination of NA + ECL lures. Therefore, not only is NA lure an inhibitor for ECL males, but ECL lure acts as an inhibitor for NA males.

Table 8. Mean number of Egyptian cotton leafworm, *Spodoptera littoralis*, males and nutgrass armyworm, *Spodoptera exempta*, males captured in traps 1/ baited with their respective pheromones alone and in traps baited with ECL pheromone in combination with various other attractants. Test was performed in Bouaké, Ivory Coast, Africa October 1-4, 1985.

Treatment (Attractant Combination) 2/ 1	2	$\bar{x}$ No. of ECL males captured/trap for 3 readings 3/	$\bar{x}$ No. of NA males captured/trap for 3 readings
ECL tre-pc	CONTROL	24.0 ab 4/	0.0 4/
ECL tre-pc	CL tre-rs	24.0 a	0.0
ECL tre-pc	CP ray-rs	13.7 bc	0.0
ECL tre-pc	CS ray-rs	19.3 ab	0.0
ECL tre-pc	EI zoe-pc	33.3 a	0.0
ECL tre-pc	NA tre-rs	6.7 c	1.7 b
CONTROL	NA tre-rs	0.0	15.7 a

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Four complete replicates were read and randomized three times for a total of twelve observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

In a third series of lure combination tests (Table 9), lures for four *Heliothis* species (*H. armigera*, *H. virescens*, *H. zea* and *H. punctigera*), when placed in traps baited for ECL, did not result in significantly lower ECL male captures. Also, the combination of ECL + GM (gypsy moth) lures did not lower ECL captures.

Table 9. Mean number of Egyptian cotton leafworm, Spodoptera littoralis, males captured in traps 1/ baited with S. littoralis pheromone alone and in paired combination with various attractants. Test performed in Bouake', Ivory Coast, Africa October 4-7, 1985.

Treatment (Attractant Combination) 2/ 1	2	$\bar{x}$ Number of Males Per Trap for 3 Readings 3/
ECL tre-pc	CONTROL	35.0 a 4/
ECL tre-pc	HA alb-fib	33.3 a
ECL tre-pc	HP alb-fib	23.3 a
ECL tre-pc	HZ her-lam	35.0 a
ECL tre-pc	HV her-lam	33.7 a
ECL tre-pc	GM her-lam	40.0 a

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Three complete replicates were read and randomized three times for nine observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

In an additional test designed to determine if red bollworm (RB) pheromone, when placed in traps baited for ECL, acts as a synergist, an additional study was designed and conducted (Table 10). To serve as controls, traps were baited with either ECL pheromone alone or RB pheromone alone. To determine the possible effect of combinations, a series of traps were baited with the lure combinations: ECL + RB, ECL + 80ug E/Z (83:17) 9, 11-Dodecadien-1-ol acetate and ECL + 200ug 11-Dodecen-1-ol acetate. The last two treatments were added to determine which RB pheromone lure components were acting as a synergist, if an effect was observed. The amounts and ratio of compounds used for this test were the same as those normally used to formulate RB lure dispensers.

Similar to the results reported in Table 7, the combination of ECL + RB lures captured more ECL males, but not a significantly greater number of males than the control treatment (ECL). Also, the numbers of ECL males captured in both treatments where ECL was combined with the components of RB lure appeared to be larger than the control. Again, however, analysis could not detect any significant difference between the combination treatments and the ECL control. Traps baited with only RB pheromone dispensers, captured no ECL males demonstrating that, alone, RB pheromone is not an attractant. If there is a synergistic effect of RB pheromone, a more extensive field test will be necessary to demonstrate and quantify the effect.

Table 10. Mean number of Egyptian cotton leafworm, *Spodoptera littoralis*, males captured in traps 1/ baited with *S. littoralis* pheromone alone and in combination with various attractants.

Treatment Attractant Combination 2/ 1	2	$\bar{x}$ Number of Males Per Trap for 3 Readings 3/
ECL her-lam	CONTROL	8.3 a 4/
ECL her-lam	RB lab-pc	15.7 a
ECL her-lam	80ug E/Z (83:17) 9,11-Dodecadien-1-ol-acetate	15.0 a
ECL her-lam	200ug 11-Dodecen-1-ol acetate	11.3 a
CONTROL	RB lab-pc	0.0 b

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Three complete replicates were read and randomized four times for twelve observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

False Codling Moth, *Cryptophlebia leucotreta*

Previously, extensive field studies have been carried out with the false codling moth (FCM) in South Africa (1984 Annual Report, Table 13, p. 125). However, because one manufacturer has changed the formulation (ie. tre-rs) for their FCM lures, a field trial was conducted to compare the three commercial formulations. For this trial, a 45 meter between trap and between replicate (line) spacing was used for trap placement. Traps were hung from stakes so that the trap was slightly above the crop height.

No significant differences could be detected in the numbers of the males captured in traps baited with the three commercial formulations. In earlier tests, the alb-fib formulation was found to be significantly better than either of the two other formulations. These earlier tests, however, were conducted over much longer periods and perhaps a more prolonged test would have revealed some differences. The change in the formulation of the tre-rs lures (formerly Zoecon-rs) has undoubtedly improved the formulation. Results of this test also demonstrated that two other *Cryptophlebia* species are attracted to FCM pheromone dispensers. Presently, we are attempting to identify these species and determine their importance as crop pests.

In a separate field trial, lures for two exotic *Chilo* species were tested for their compatibility in FCM traps. Trap spacing and placement was the same as in the previous test. Results of this test are presented in Table 12. These data demonstrate that the additions of either *Chilo partellus* (CP) or *Chilo suppressalis* (CS)pheromones to traps baited for FCM does not inhibit FCM male capture.

Table 11. Mean numbers of false codling moth, *Cryptophlebia leucotreta*, males captured in traps 1/ baited with various commercial formulations of *C. leucotreta* pheromone. Test performed in Bouake', Ivory Coast, Africa September 23-26, 1985.

Treatment 2/	$\bar{x}$ Number of Males Captured Per Trap for 3 Readings 3/
FCM alb-fib	53.00 a 4/
FCM tre-rs	66.25 a
FCM hoe-rt	70.75 a

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Four complete replicates were read and randomized three times for twelve observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Table 12. Mean number of false codling moth, Cryptophlebia leucotreta, males captured in traps 1/ baited with C. leucotreta pheromone alone and in paired combination with various attractants. Test performed in Bouaké, Ivory Coast, Africa September 26-30, 1985.

Treatment (Attractant Combination) 2/ 1	2	$\bar{x}$ Number of Males Per Trap for 3 Readings 3/
FCM tre-rs	CONTROL	64.0 a 4/
FCM tre-rs	CP ray-rs	72.8 a
FCM tre-rs	CS ray-rs	68.3 a

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Four complete replicates were read and randomized twice for eight observations per treatment.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Nutgrass armyworm, Spodoptera exempta

Although populations of nutgrass armyworm (NA) adults were sparse, a preliminary study was initiated to determine which trap design was most efficient in capturing NA males. For testing, four trap designs were baited with the same formulation of NA pheromone. Four complete replicates (lines) of the test were established in rice fields with a 30 m between line and between trap spacing. For placement, traps were suspended from stakes so that they were just above crop height.

Results of this trap design test, reported in Table 13, are inconclusive. Although the test was conducted over a two week period, numbers of NA males captured were too small to provide statistical resolution. If any information can be gained from the data, it is that traps with restrictive entry ports (ie. the delta trap with the end closed "or-delta-ec) should not be used for survey.

Table 13. Mean numbers of nutgrass armyworm, *Spodoptera exempta*, males captured in traps of various designs baited with S. exempta pheromone.1/ Test performed in Beheke, Ivory Coast, Africa September 23 - October 7, 1985.

Trap Type 2/	$\bar{x}$ Number of Males Captured Per Trap for 3 Readings 3/
or-delta-ec	0.5 a 4/
or-delta-e.o.	3.5 a
Inter-delta	3.0 a
P-1C	1.25 a

1/ All traps baited with a commercial formulation of *S. exempta* pheromone (TRECE).
2/ See Appendix 4 for trap types.
3/ Four complete replicates were read and randomized three times for a total of twelve readings per treatment.
4/ Means followed by the same letter are not significantly different according to Analysis of Variance for a randomized complete block design. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Pink bollworm, *Pectinophora gossypiella*

Four lure formulations for the pink bollworm were tested in a small field trial. Traps, suspended from stakes at the cotton crop height, were placed 45 m apart along lines (replicates). In all, four lines of traps were established with a 45 m spacing. Results of this test, presented in Table 14, demonstrate that the USDA (lab-rs) and Hoechst (hoe-rt) formulations of PBW pheromone captured significantly more males than either of two other commercial formulations.

Table 14. Mean numbers of pink bollworm, Pectinophora gossypiella, males captured in traps 1/ baited with various formulations of P. gossypiella pheromone. Test performed in Bouaké, Ivory Coast, Africa September 23-30, 1985.

Treatment 2/	$\bar{x}$ Number of Males Captured Per Trap 3/
PBW her-lam	16.25 a 4/
PBW alb-fib	17.00 a
PBW lab-rs	40.00 b
PBW hoe-rt	33.00 b

1/ Wing type Pherocon-1C traps used for all treatments.
2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
3/ Four complete replicates for all treatments except PBW hoe-rt (2 replicates) were read and randomized three times.
4/ Means followed by the same letter are not significantly different at the 5% level according to Analysis of Variance. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Old world bollworm, Heliothis armigera

Earlier tests with Heliothis armigera (HA), in Australia, indicated that one of two commercial formulations tested was superior for trapping this species (1984 Annual Report, Table 14, p. 127). A formulation which resulted in significantly fewer moth catches was the formulation standardly used by the Australian Dept. of Primary Industries for their HA surveys. To determine which of these two formulations, previously tested, or three other formulations was superior for trapping HA males, a field test was established in cotton fields in Bouake'. Three replicates of the test were read daily and rotated between locations weekly. For placement, traps were suspended from stakes at the crop height.

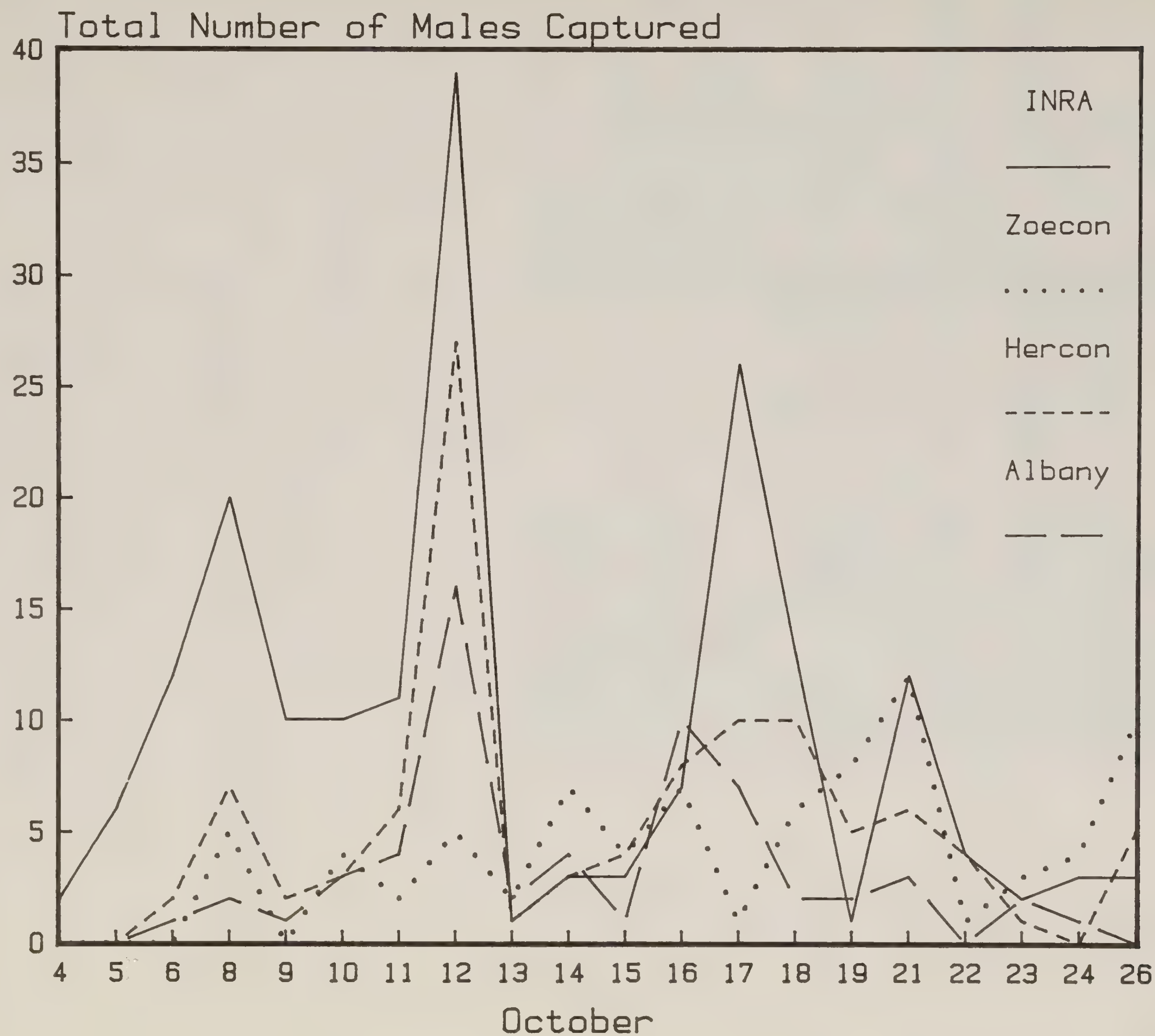
Results of this trial demonstrate that the INRA formulation for HA pheromone lures is clearly superior and would be the lure of choice if domestic survey was undertaken for this species. Unlike earlier tests in Australia, no clear differences could be detected between the alb-fib formulation and the her-lam formulation. Clearly, the hoe-pc formulation is deficient; very few HA were captured, yet several Earias biplaga, a non-target species, were captured by only this formulation. The zoe-rs formulation not only captured significantly fewer males than the INRA-rs formulation, but the period in which males are captured appears to be different than three of the other formulations (Figure 1.). Traps baited with the INRA-rs, her-lam and alb-fib formulations capture curves appear to agree, whereas traps baited with the zoe-rs formulation captured few males early in the flight period and larger numbers when the flight period was nearly over.

Table 15. Mean numbers of Old world bollworm, Heliothis armigera males captured in traps 1/ baited with five commercial formulations of H. armigera pheromone. Test was conducted in Bouake', Ivory Coast, Africa, 10/4/85 - 10/26/85. 2/

Treatment 2/	$\bar{x}$ Number of Males Captured Per Trap 3/
HA INRA-rs	62.7 a
HA zoe-rs	27.0 b
HA her-lam	34.7 b c
HA alb-fib	20.3 c
HA hoe-pt 5/	0.7 d

1/ Wing type Pherocon-1C traps were used for all treatments.
2/ Three complete replicates were read daily and moved to new locations weekly.
3/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2 and 3, respectively.
4/ Means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range test. Analysis performed on transformed data [log(n+1)]; actual means are presented.
5/ A total of thirteen Earias biplaga were captured in traps baited with the Hoechst formulation. This non target was not captured in traps baited with the other formulations.

Fig. 1 Numbers of H. armigera males captured daily. Traps baited with four formulations of pheromone.



Appendix 1. Species codes - scientific names - common names.

ADOX	*Adoxophyes orana*	summer fruit tortrix
AV	*Argyrotaenia velutinana*	red banded leafroller
CL	*Spodoptera litura*	cotton leafworm
CM	*Laspeyresia pommonella*	codling moth
CP	*Chilo partellus*	maize borer
CS	*Chilo suppressallis*	Asiatic rice borer
EA	*Clysia (Eupoecilia) ambiguella*	European grape berry moth
ECL	*Spodoptera littoralis*	Egyptian cotton leafworm
EI	*Earias insulana*	spiny bollworm
EP	*Epiphyas postvittana*	light-brown apple moth
FCM	*Cryptophlebia leucotreta*	false codling moth
GM	*Lymantria dispar*	gypsy moth
GP	*Grapholita prunivora*	lesser apple worm
HA	*Heliothis armigera*	Old World bollworm
HP	*Heliothis punctigera*	Australian bollworm
HV	*Heliothis virescens*	tobacco budworm
HZ	*Heliothis zea*	corn earworm
LB	*Lobesia botrana*	European grapevine moth
NA	*Spodoptera exempta*	nutgrass armyworm
OFM	*Grapholita molesta*	Oriental fruit moth
PBW	*Pectinophora gossypiella*	pink bollworm
PFM	*Cydia funebrana*	plum fruit moth
PS	*Pectinophora scutigera*	pink-spotted bollworm
PV	*Paralobesia viteana*	grape berry moth
RB	*Diparopsis castanea*	red bollworm
TAB	*Platynota idaeusalis*	tufted apple bud moth

Appendix 2. Manufacturer codes:

alb	Albany International
her	Health-Chem Corporation
hoe	Hoechst
INRA	French, Institut National de la Recherche Agronomique
lab	prepared by Otis Methods Development Center
ray	Raylo Chemical Corp.
tre	TRECE
zoe	Zoecon Corporation

Appendix 3. Dispenser type codes:

fib	hollow micro fibers
lam	3 layer plastic laminate
pc	poly caps
pt	plastic tube
rs	rubber septa
rt	rubber tube

Appendix 4. Trap codes:

delta — USDA delta trap
- Prefixes:
  - or = orange
  - br = brown
- Suffixes:
  - eo = end panels of trap not folded in (open)
  - ec = end panels folded in normal position (closed)

Hoe-delta — delta type "biotrap" manufactured by Heochst

INRA-delta — large delta trap used by I.N.R.A.
- Suffixes:
  - w.o. = side window panels open

Inter-delta — large delta trap (similar to INRA trap) manufactured by International Pheromone Systems. Does not have side window panels.

M.C. — high capacity USDA gypsy moth trap (milk carton)

Multi-1 — Multi-pher - a bucket type trap (20cm h x 34cm cir) with four top entry ports (8.2cm wide x 2.5cm h). Vaponna is used to kill moths entering the trap. Distributed by Les Services Bio-Condrole.

Multi-2 — Multi-pher - a bucket type trap (20cm h x 34cm cir) with ten top entry ports (2.5cm wide x 0.8cm h). Vaponna is used to kill moths entering the trap. Distributed by Les Services Bio-Condrole.

P-1C — Pherocon-1C "wing type" trap

1985 - PILOT SCALE SURVEY

In 1985, pilot scale survey was undertaken for six new exotic pests.

Silver Y moth	Autographa gamma
Maize borer	Chilo partellus
Asiatic rice borer	Chilo suppressalis
Light brown apple moth	Epiphyas postvittana
European grape berry moth	Eupoecilia ambiguella
Cabbage moth	Mamestra brassicae

The objectives of this pilot study were to determine what logistical problems were involved in conducting a national survey and, more importantly, to identify potential problems with non-target organisms in traps.

A summary of the number of traps provided to each state for each species is presented on Table 16. A total of 370 traps were shipped for the six species to mostly APHIS field locations. A total of twenty states cooperated in this pilot scale survey. Of the states reporting results, (Table 17) information on non-target insects was obtained on five of the six target exotic species (Tables 18 through 23). Because of the small amount of data which had been reported at the time this summary was prepared, a full discussion of the results will not be attempted. When remaining results are reported, this project will be discussed in detail.

Table 16. Number of pheromone traps provided for use in 1985 pilot scale exotic pest survey.

State Code	Autographa gamma	Chilo partellus	Chilo suppressalis	Epiphyas postvittana	Eupoecilia ambiguella	Mamestra brassicae
AK		15	20		5	
CA	5	5	5	5	5	5
FL	5			17		
KY		25				
LA		15	15			
MD	5					
MI	5				5	8
MO			8		5	
MS		5				
NJ	5					
NY	5				5	5
NC	5	5		5	5	
NB	10					
NM	10					
OH					15	
OR	5				5	8
PA	5				5	
SC		5				
TN		5	19			2
TX	5	20	15			8
Totals	70	100	82	27	55	36

Table 17. Number of pheromone traps placed in the 1985 pilot scale exotic pest survey program from which data were obtained.

State Code	Autographa gamma	Chilo partellus	Chilo suppressalis	Epiphyas postvittana	Eupoecilia ambiguella	Mamestra brassicae
AK		29				
MS		2				
NY	5				11	5
NB	13					
NM	7					
OH	5				10	
OR	5			17		8
TX	5					

Table 18. Total number of non-target insects captured in traps baited for *Autographa gamma*.

State	No. of Traps	Species Codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14
NE	13	66											21		
NM	7	77													
NY	5	11					34	33	1	164	2	11			16
OH	5	86													
OR	5		16	22	32	4									
TX	5												4	3	

Insect Codes:

1. Lepidoptera:Noctuidae *Syngrapha falcifera*
2. Lepidoptera:Pieridae *Pieris rapae*
3. Lepidoptera:Noctuidae *Lacanobia lutra*
4. Lepidoptera:Noctuidae *Autographa californica*
5. Lepidoptera:Noctuidae *Polias* spp.
6. Lepidoptera:Pterophoridae *Geina periscelidactyla*
7. Lepidoptera:Noctuidae *Anagrapha ampla*
8. Lepidoptera:Trotricidae *Episemus argutanus*
9. Lepidoptera:Pyralidae *Helvibotys helvialis*
10. Lepidoptera:Pyrlaidae spp.
11. Lepidoptera:Gelechliidae spp.
12. :Noctuidae *Rachiphusia ou*
13. Lepidoptera:Noctuidae *Pseudoplusia includens*
14. Lepidoptera:Lymantriidae *Lymantria dispar*

Table 19. Total number of non target insects captured in traps baited for *Chilo partellus*.

State	No. of Traps	Species Codes 1
AK	29	238
MS	2	51

Insect Codes:

1. Lepidoptera:Ctenuchidae (=*Amatidae*) *Cisseps fulvicollis*

Table 20. Total number of non target insects captured in traps baited for *Chilo suppressalis*.

No States Reporting

Table 21. Total number of non-target insects captured in traps baited for *Epiphyas postvittana*.

State	No. of Traps	Species Codes 1	2	3
OR	17	4,029	48	56

Insect Codes:

1. Lepidoptera:Gradillariidae *Phyllonorycter spp*.
2. Lepidoptera:Pyralidae *Pyrausta rubricalis*
3. Lepidoptera:Tortricidae *Archips rosaceana*

Table 27. Total number of non-target insects captured in traps baited for *Clysia* (=*Eupoecilia*) *ambiguella*

State	No. of Traps	Species Codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14
NY	11	1,372	55		1	83	17	2	22	47	1	1	23	4	3
OH	10	751	19	16											

Insect Codes:

1. Lepidoptera Tortricidae *Episemus argutanus*
2. Lepidoptera Tortricidae *Endopiza viteana*
3. Lepidoptera Tortricidae *Argyrotaenia velutinana*
4. Lepidoptera Oecophoridae *Agonopterix pulvitennella*
5. Lepidoptera Tortricidae *Phaneta crispana*
6. Lepidoptera Tortricidae *Pseudogalleria inimicella*
7. Lepidoptera Tortrididae *Ptycholoma teritana*
8. Lepidoptera Lymantriidae *Lymantria dispar*
9. Lepidoptera *Grapholithua earyma*
10. Lepidoptera Noctiduae *Autographa precationis*
11. Lepidoptera Noctiduae *Faronta diffusa*
12. Lepidoptera Gelechiidae *Phthorimaea operculella*
13. Lepidoptera Tortricidae *Grapholitha prunivora*
14. Lepidoptera Geometridae *Eusarca confusara*

Table 23. Total number of non-target insects captured in traps baited for *Mamestra brassicae*.

State	No. of Traps	Species Codes 1	2	3	4	5	6	7	8	9	10	11	12
NY	5					30	11	4	66	11	1	92	1
OR	8	33	46	48	5								

Insect Codes:

1. Lepidoptera *Pieridae* *Pieris* *rapae*
2. Lepidoptera Noctuidae *Lacanobia* *lutra*
3. Lepidoptera Noctuidae *Autographa* *californica*
4. Lepidoptera Noctuidae *Polias* spp.
5. Lepidoptera Noctuidae *Faronta* *diffusa*
6. Lepidoptera Noctuidae *Aletia* *oxygala*
7. Lepidoptera Noctuidae *Pseudaletia* *unipuncta*
8. Lepidoptera Noctuidae *Polia* *detracta*
9. Lepidoptera Noctuidae *Scotogramma* *trifolii*
10. Lepidoptera Noctuidae *Abrostola* *urentis*
11. Lepidoptera Noctuidae *Orthodes* *crenulata*
12. Lepidoptera Lymantriidae *Lymantria* *dispar*

EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS FOR 1986

This package contains guidelines for survey for thirteen exotic insect species. Included are 1) General Trapping Guidelines, 2) Methodology for removing insects from traps, 3) specific information on each target species and 4) identification aides.

Traps and baits will be available for the following fourteen exotic pests in 1986. Listed are approved common and scientific names and EPA codes.

Scientific Name	Common Name	Otis MDC Pheromone Code	EPA Code
Adoxophyes orana	Summer fruit tortrix moth	ADOX	ITBUETA
Autographa gamma	Silver Y moth	AB	ITBCFCA
Chilo partellus	Maize borer	CP	ITBMEVA
Chilo suppressalis	Asiatic rice borer	CS	ITBMADA
Cryptophlebia leucotreta	False codling moth	FCM	ITBUEUA
Cydia funebrana	Plum fruit moth	PFM	ITBUESA
Epiphyas postvittana	Light brown apple moth	LBAM	ITBUBPA
Eupoecilia (=Clysia) ambiguella	European grape berry moth	EA	ITCLABA
Lobesia botrana	Grape vine moth	LB	ITBUEVA
Mamestra brassicae	Cabbage moth	MB	ITBCDMA
Rhagoletis cerasi	European cherry fruit fly	RC	IOBMCDA
Spodoptera littoralis	Egyptian cottonworm	ECL	ITBCFPA
Spodoptera litura	Rice cutworm	CL	ITBCFMA
Trogoderma granarium	Khapra beetle	KB	INATANA

USDA, APHIS, Plant Protection and Quarantine will provide traps and pheromone dispensers for these species and procurement will be coordinated through PPQ regional offices. If there are specific questions concerning traps, trap placement, trap servicing or pheromone dispensers, please contact Otis Methods Development Center (617) 563-9303.

The information in this manual was compiled by the Otis Methods Deveopment Center with inputs from the Survey and Emergency Response Staff and the Insect Identification and Beneficial Insect Introduction Institute, ARS.

Otis Methods Development Center - 11/27/85

GENERAL TRAPPING GUIDELINES

Careful preparation and handling of traps and baits is an important part of conducting a productive detection survey. Traps should be assembled according to instruction provided, giving particular attention to critical dimensions (e.g. entry port openings). Damaged traps that have tack-trap on the outside surfaces or do not have enough tack-trap on the inside (catching surfaces) should be discarded or returned. Baits (pheromone dispensers) should be handled carefully to prevent contamination of the outside trap surfaces or cross contamination with other baits. Careful handling of baits with forceps or disposable rubber gloves should prevent contamination of the trapper and the traps. Forceps should be cleaned and gloves should be changed when switching bait types. Preparing all trap components first, and subsequently baiting these traps with lures, should also minimize contamination of the outer trap surfaces.

Baits should be placed in traps so that when traps are serviced the bait can easily be moved to a new trap or, in the case of some trap types (wing-type traps), the bait should be attached to the portion of the trap that does not require replacement (i.e. underside of the roof of wing-type traps). Under no circumstances should baits be placed in the adhesive (i.e. tack-trap). Placement of polycap type bait dispensers (small plastic vials) is facilitated by wrapping a piece of thin copper wire around the hinge of the dispenser. The trailing ends of the wire can then be stapled to the trap interior. Do not open polycap dispensers. Other pheromone dispenser types (rubber septa, hollow fibers, and laminates) can simply be stapled centrally in the trap.

Unused traps should be stored in a cool, dry area that is free of pheromone dispensers. Baits should be stored in tightly sealed glass containers in a freezer. Again, care should be exercised so that different bait types are not mixed in the same container (which would result in cross contamination). Bait dispensers and containers of baits should not be exposed to strong light for long periods of time. Some pheromone components are photosensitive and will degrade rapidly if left in bright light.

Traps should be serviced as often as possible. Frequent checking will prevent trap loading and will facilitate identification of trapped specimens. It is suggested that traps be checked at least every two weeks (at a minimum) unless other conditions suggest more frequent checking is necessary (i.e. traps overload or replacement of pheromone dispensers is required). Guidelines for handling collected specimens is covered in a separate section of these recommendations, Appendix 1. Volatility and degradation rates vary between pheromone components among the various species, and release characteristics are different for the different types of dispensers. For these reasons, no generalizations can be made about field life of baits. The expected field life and recommended intervals for bait replacement are listed for each individual species.

Placement of traps (i.e. height, crop) is also outlined for each individual species. Flight characteristics and responses to pheromones vary from species to species; closely following these recommendations will maximize trap efficency. Little or, in most cases, no work has been done on the optimal trap spacing for detection of these exotic pests. However, information is available about flight ability and response to pheromone over distances.

Given this information, the best approach is to place traps over as large a geographic area as feasible while optimizing the location of each individual trap. Where possible, traps should be placed within the host crop of the target insect. Crops which are not sprayed will likely harbor larger populations of the target species and placement at these sites will enhance detection. When trapping two species with the same trap, placement of the trap where host plants are adjacent or in close proximity will be the most desirable location. Recommendations for combinations are listed under each individual species. When traps are hung within a crop, care should be exercised so that entry ports are not blocked by vegetation or, in some cases, blocked by the stake the trap is hung on. When servicing traps, any damaged traps, or traps that have the sticky surface saturated with insects, dirt or debris, should be replaced.

PROCEDURES FOR IDENTIFICATION AND REMOVAL OF
INSECT SPECIMENS FROM STICKY TRAPS

Removal of insect specimens from sticky traps in a condition suitable for subsequent examination with a microscope is sometimes desirable. This can be done with little difficulty for many groups of insects, particularly those with hard exoskeletons, such as beetles and wasps. Successful removal of soft-bodied or scaly insects, such as aphids and caddisflies, is more difficult and is virtually impossible with certain groups, such as moths. Moths cannot be removed without seriously damaging the scale patterns which are the characters generally used as the first step in identification. For moths, it is best to attempt identification, or provide the identifier, with the specimen(s) in place on the sticky trap (either the entire trap or the part bearing the moth(s) being cut out). If identification cannot be made in this way, it may be appropriate to dissolve the moths from the sticky material and examine the genitalia. Such specimens may be identified by examining the abdomen, i.e., the genitalia, which can be carefully removed, cleaned according to the following methods, and preserved.

Polyisobutylene is the most widely used sticky material in sticky traps. This material is non-polar and is thus poorly dissolved by a polar solvent such as acetone. Effective and preferred materials for specimen removal are toluene, heptane, hexane, xylene, and ethyl acetate, all of which can be readily obtained. Solvents for occasional use and which are also readily available are fingernail polish remover (ethyl acetate) and a solvent-cleaner, methyl chloroform (1,1,1-trichloroethane) (the current replacement for carbon tetrachloride). Petroleum spirits is effective but leaves a short-term residue, whereas gasoline or kerosene will linger on the specimens for days or weeks and are not preferred. SINCE ALL OF THESE SOLVENTS ARE FLAMMABLE AND ARE TOXIC TO HUMANS TO SOME EXTENT, THEY SHOULD BE HANDLED OUTDOORS OR UNDER A HOOD.

A decision must be made as to the number of specimens to be removed from the sticky trap. If a general survey is intented, the entire trap may be immersed in the solvent until the sticky material is dissolved. The solvent is then drained off, leaving the intact insects behind for further treatment as described below. If only a few insects are to be removed, selected pieces of the trap may be cut out and immersed in the solvent to free the specimens. <u>In each method, the insects should be immersed until free of the sticky material but no longer, as the solvents have a tendency to make the specimens brittle.</u>

After all of the sticky material has been dissolved from the specimens, they must be washed in Cellosolve^{R} and then xylene to remove the solvent. Immerse the specimens in a bath of Cellosolve for an hour or longer to remove the solvent, replacing the Cellosolve after half an hour if many specimens are processed simultaneously. The specimens may safely be left overnight in Cellosolve. The Cellosolve should then be drained off and replaced with xylene for one-half to one hour. <u>Caution: all insects become brittle and some are permanently damaged by prolonged immersion in xylene</u>. The specimens may then be placed on absorbent paper and dried. If they are manipulated with fine insect pins or a camel's hair brush while drying, the wings, body hairs and bristles will assume their natural positions. The specimens may then be carefully point-mounted or glued to the side of a pin and labelled.

EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Adoxophyes orana   Summer fruit tortrix moth

Hosts: Apple, Pear

Distribution: See map

Biology: Adoxophyes orana is a bivoltine tortricid which overwinters as 2nd or 3rd instar larvae in bud axiles, bark crevices and under dry leaves. Diapause is induced by short (less than 12-hour) day length. In the Netherlands, diapause is normally initiated in late September or early October. Diapausing larvae can survive the low winter temperature of Northern Europe.

Overwintering larvae begin feeding in the spring after an accumulation of approximately 67 degree-days C (ddC) (in Romania) based on a 9°C developmental threshold. These larvae feed on the leaves and flowers of the host plants and pupate in May. In France, adult moths emerge during the first part of June, with oviposition shortly thereafter. Second generation adults emerge in mid-August. Flight occurs at temperatures above 13°C. The summer generation of larvae lasts, on average, 430 ddC above a threshold of 7°C in France, and feed mainly on the leaves. Second generation larvae feed on fruit before entering diapause in the fall.

Up to 10% and 20% fruit loss has occurred in France and Germany, respectively.

Potential U.S. distribution: Throughout the US, wherever host plants occur (see map).

Recommended survey areas: Major apple and pear producing areas (see map). WA, NY, MI, CA, PA, VA, NC, WV, OR, NJ, IL, MA, ME, ID, CO, MD, OH, MO, NH, WI, IN, UT, VT, CT

Pheromone: 90:10 mixture of (Z)-9:(Z)-11-tetradecenal acetate
dispenser type - polycap
field life - 3 months, if a longer trapping period than 3 months is anticipated, replace baits at midseason.

Commercial source of pheromone dispensers: United Agri Products Co., Trece Corp.

Traps: USDA - delta trap - ends open (i.e. the ends of the trap which are normally folded in to form a small triangular entry port, should not be folded); Wing Trap

Trap placement: Within apple or pear orchards, suspended from the limbs of trees ca. 1.5 m in height.

Recommended combinations: None presently recommended. A. orana pheromone is, in fact, a powerful inhibitor to many other species. Contamination of other traps should be carefully avoided.

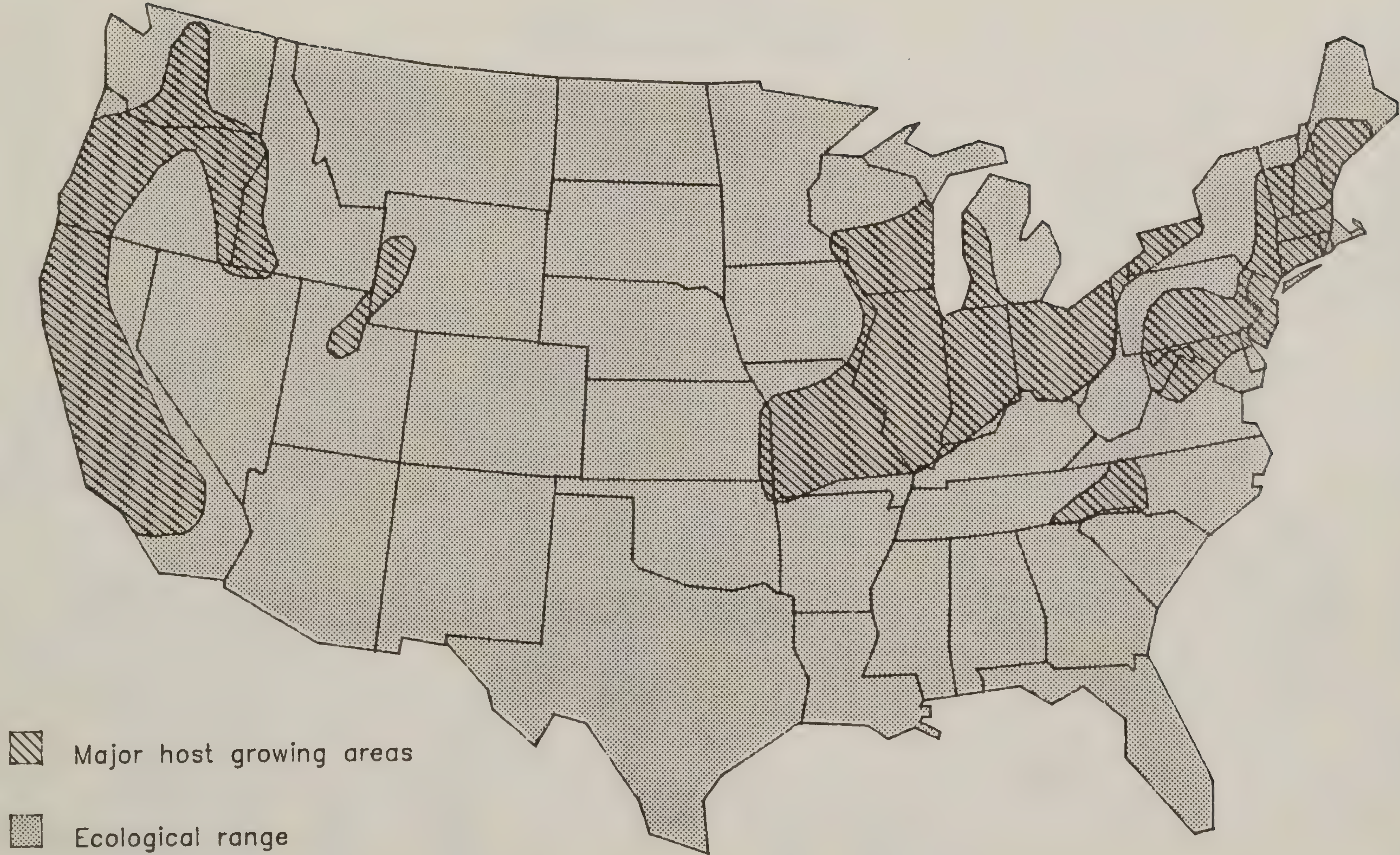
Non-target species that may be captured: *Choristoneura rosaceana*, *Pandemis limitata*, *Grapholita molesta*, *Argyrotaenia velutinana*, *Pandemis pyrusana*.

Otis Methods Development Center 11/27/85

Adoxophyes orana

World distribution

Adoxophyes orana



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Autographa gamma Silver Y moth

Hosts: Most cultivated crops including potatoes, beets, peas, crucifers cereals, etc.

Distribution: See map

Biology: Eggs are laid either singly or in small clusters on the underside of leaves. Larvae feed on leaves and pupate in off-white cocoons on the host plant. There are usually two generations per year with overwintering in the cocoon.

Potential U.S. Distribution: Throughout the U.S.

Recommended Survey Areas: Because this pest can cause severe damage on many crops and can potentially occur throughout the U.S., we are not designating specific states to be surveyed but suggest trapping in major truck farming areas.

Pheromone: 100:1 mixture of (Z)-7-Dodecen-1-ol acetate:(Z)-7-Dodecen-1-ol
loading rate - 1.0 mg
dispenser type - rubber septa
field life - 30 days - replace baits every 30 days.

Source of Pheromone Dispensers: Otis Methods Development Center.

Traps: United Agri Products and Trece Wing Traps

Trap Placement: Within or on the edge of fields of host crops. Traps should be suspended from stakes and placed at the level of the crop height and raised as the crop matures.

Recommended Combinations: None presently recommended.

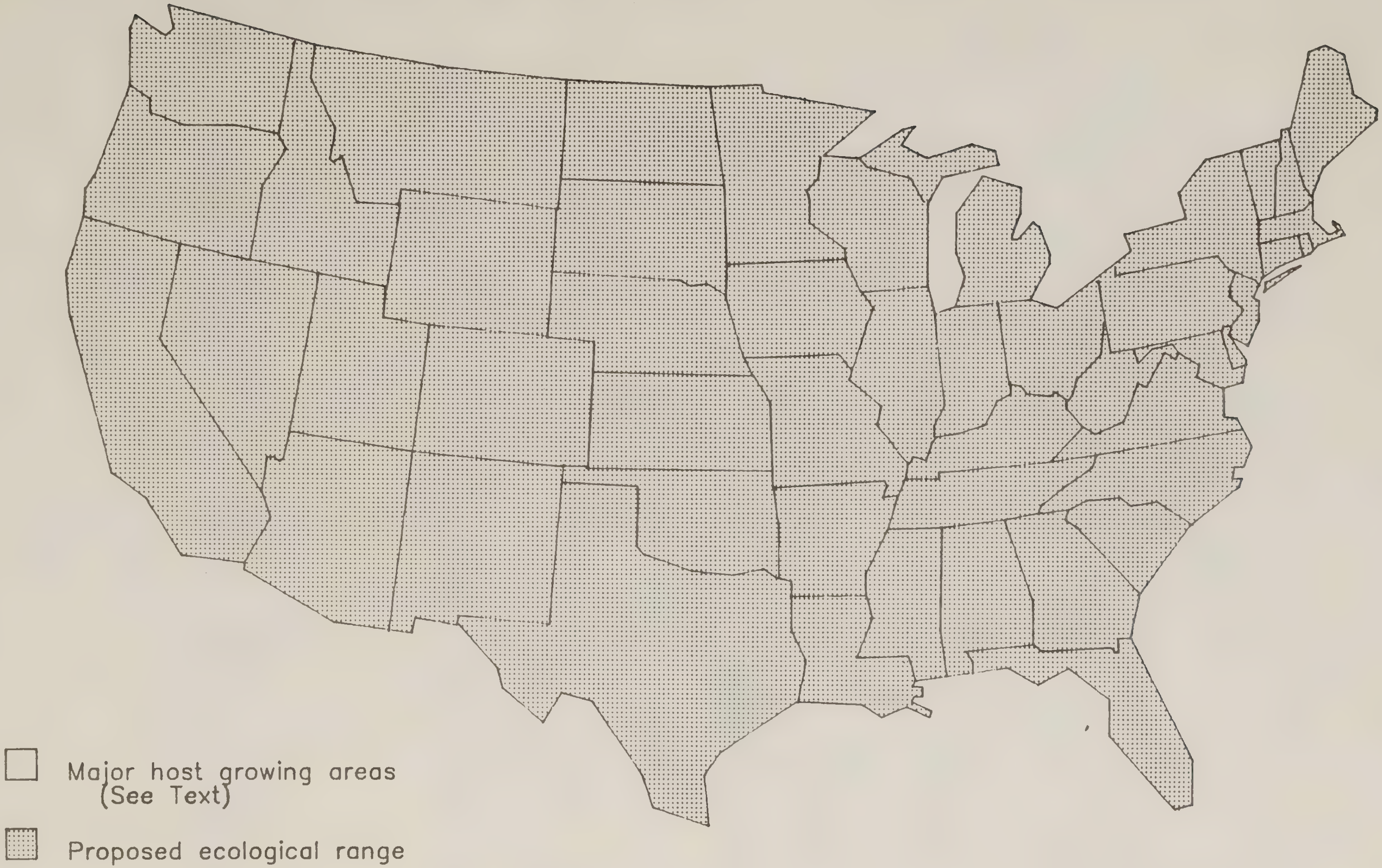
Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic non-targets is unknown.

Otis Methods Development Center - 11/27/85

Autographa gamma

World distribution

Autographa gamma



Chilo partellus Maize borer

Hosts: Corn, sorgum

Distribution: See map

Biology: Adult moths are nocturnal and lay eggs near the leaf-base. Small larvae feed on the leaf whorl or mine leaves, while later instars bore into the stalks or ears. Pupation takes place in the stems or stalks. This species is multivoltine with up to seven generations per year in India. Mature larvae overwinter in stalks, stubble or in the ears of corn.

Potential U.S. Distribution: *

Recommended Survey Areas: Major corn and sorgum producing states within the proposed ecological range of pest. CA, AZ, TX, LA, MS, AL, GA, SC, NC

Pheromone: (Z)-11-Hexadecenal
loading rate - 500 ug + 500 ug BHT (antioxidant)
dispenser type - rubber septa "extracted" or polyvials.
field Life - 30 days - replace baits every 30 days.

Sources of Pheromone Dispensers: Raylo Chemicals Ltd., Otis Methods Development Center.

Traps: United Agri Products and Trece Wing Trap

Trap Placement: Within fields of host crops. Traps should be suspended from stakes just below the crop height and raised as the crop matures.

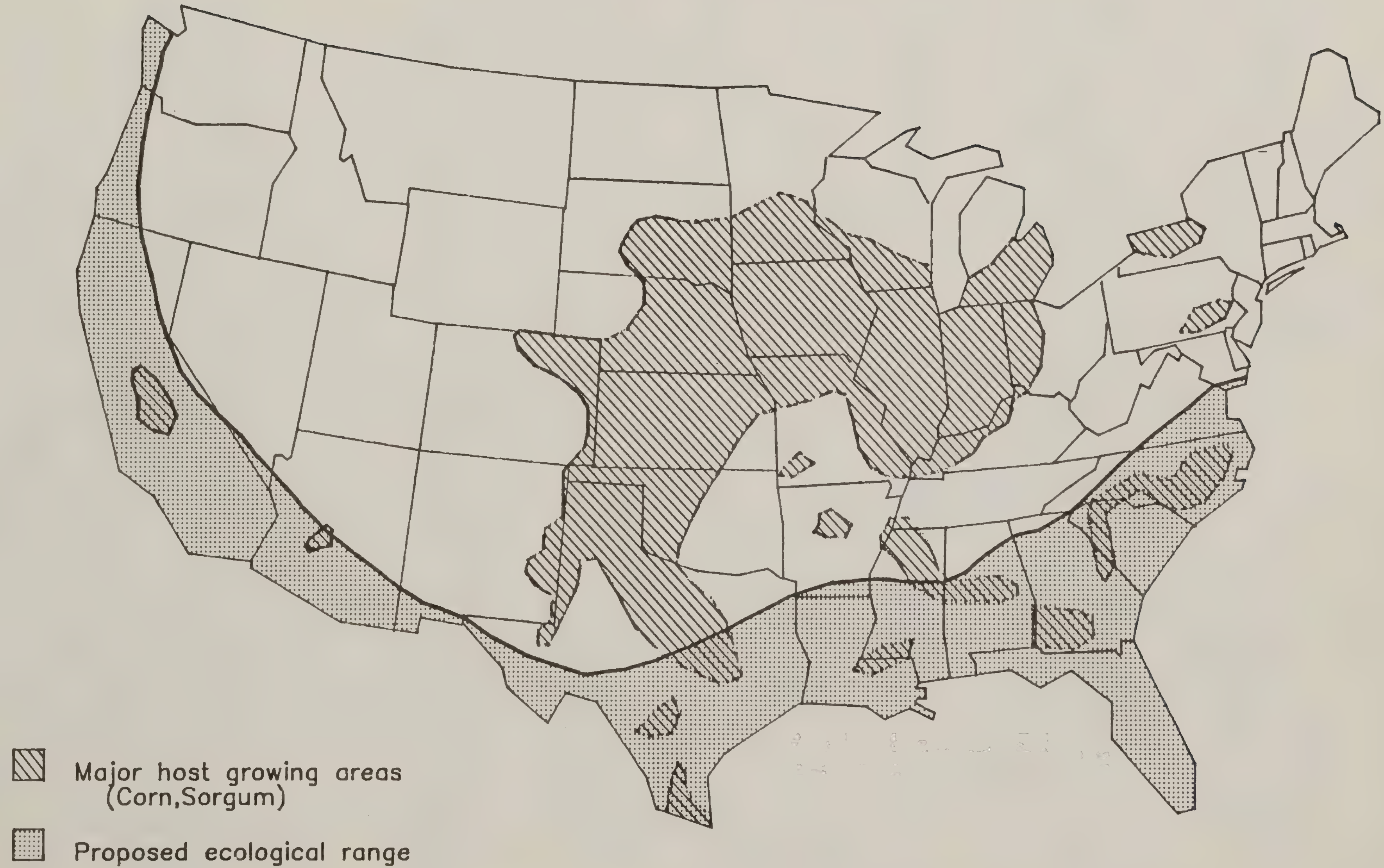
Recommended Combinations: None

Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic non-targets is unknown.

* The potential ecological range of this pest is in question since information regarding its northern limit is presently lacking or confused because of taxonomic problems. The proposed map is simply a "best guess" until more data are available.

Otis Methods Development Center - 11/27/85

Chilo partellus



Chilo suppressalis Asiatic rice borer

Hosts: Several grasses including corn but rice is most severely damaged.

Distribution: See map

Biology: The biology of this moth is similar to that of *C. partellus*. Adults are nocturnal and lay eggs in clusters on the leaf undersurface. Young larvae often aggregate when first feeding on leaves then enter the stem at the point of leaf attachment. The number of generations per year varies from one, in northern Japan and Manchuria, to seven in southwest China and Africa. Mature larvae overwinter in stalks.

Potential U.S. Distribution: Throughout the U.S. wherever host plants occur.

Recommended Survey Areas: Major rice growing areas (see map). AR, LA, TX, CA, MS, MO.

Pheromone: 75.2:16.5:8.3 mixture of (Z)-11-Hexadecenal, (Z)-13-Octadecenal and (Z)-9-Hexadecenal.
loading rate - 109 ug + 109 ug BHT (andioxidant)
dispenser type - Rubber septa "extracted"
field life - 30 day - replace baits every 30 days.

Sources of Pheromone Dispensers: Raylo Chemicals Ltd., Health-Chem Corp., and Otis Methods Development Center

Traps: United Agri Products and Trece Wing Traps

Trap Placement: Within fields of host crops traps should be suspended from stakes just below the crop height and raised as the crop matures.

Recommended Combinations: None presently recommended.

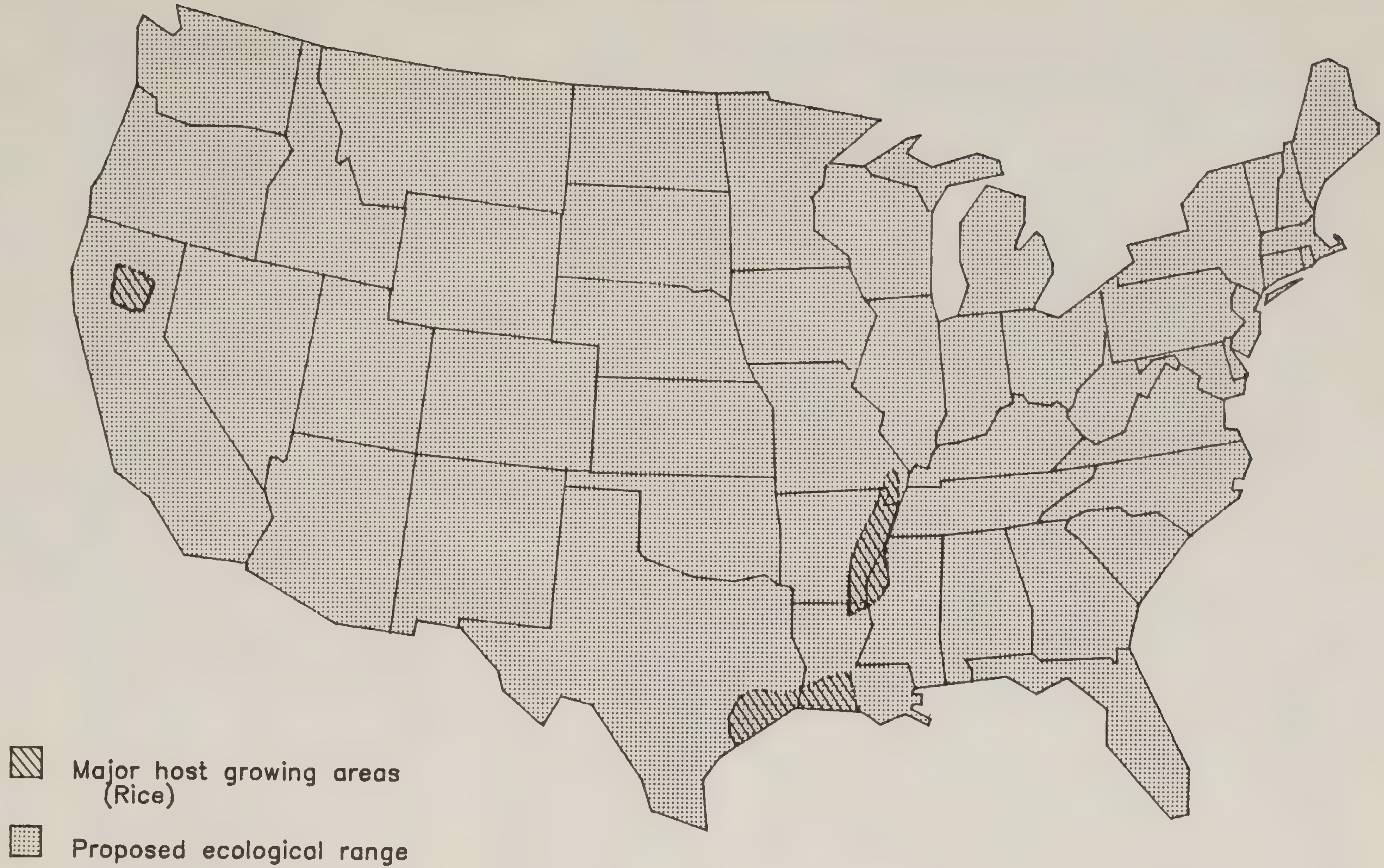
Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic non-targets is unknown.

Otis Methods Development Center - 11/27/85

Chilo suppressalis

World distribution

Chilo suppressalis



Cryptophlebia leucotreta   False codling moth

Hosts: Citrus, Cotton, Sorgum, Corn, Peach, Oak, etc.

Distribution: See map

Biology: This tortricid is multivoltine with up to six generations annually in S. Africa, where it breeds throughout the year on oranges. Depending on temperature, it can complete a generation in 45-100 days. There was no mention of a diapause in the literature reviewed. Females fly at night and usually deposit about 150-200 eggs, beginning 2-3 days after emergence. Eggs are laid on the leaves and bolls of cotton and on the fruit of citrus, and hatch in 4-14 days. Eight days of temperatures below 1.1°C is lethal to the eggs, however high mortality will also occur at 13°C and 30 percent relative humidity. The developmental threshold for eggs is 11.9°C. Larvae feed in the fruit and bolls and then drop to the soil surface to pupate. Twenty-one days of temperatures below -0.6° is lethal to larvae, and prepupal and pupal mortality is high at average ambient temperatures of 10.5°C and below.

Although this species has a wide host range, apparently it is of greatest economic importance on citrus and cotton, which have suffered major losses in Africa.

Potential U.S. Distribution: Where the average annual minimal temperatures are above -10°C (see map).

Recommended survey area: Major citrus and cotton growing areas (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL

Pheromone: 50:50 mixture of (Z):(E)-8-dodecenyl acetate
dispenser type - strips of hollow fibers
field life - 8 weeks, replace bait midseason or every 8 weeks, which ever time period is shorter

Commercial source of pheromone dispensers: United Agri Products; Trece Corp.

Traps: United Agri Products, Trece Corp. (Wing Trap)

Trap placement: In citrus and peach orchards traps should be suspended from the tree limbs at ca. 1.5 meters in height. In row crops, traps should be placed on stakes at the same height as the crop.

Recommended combinations: None presently recommended. (*Pectinophora scutigera* is compatible with *C. leucotreta*.)

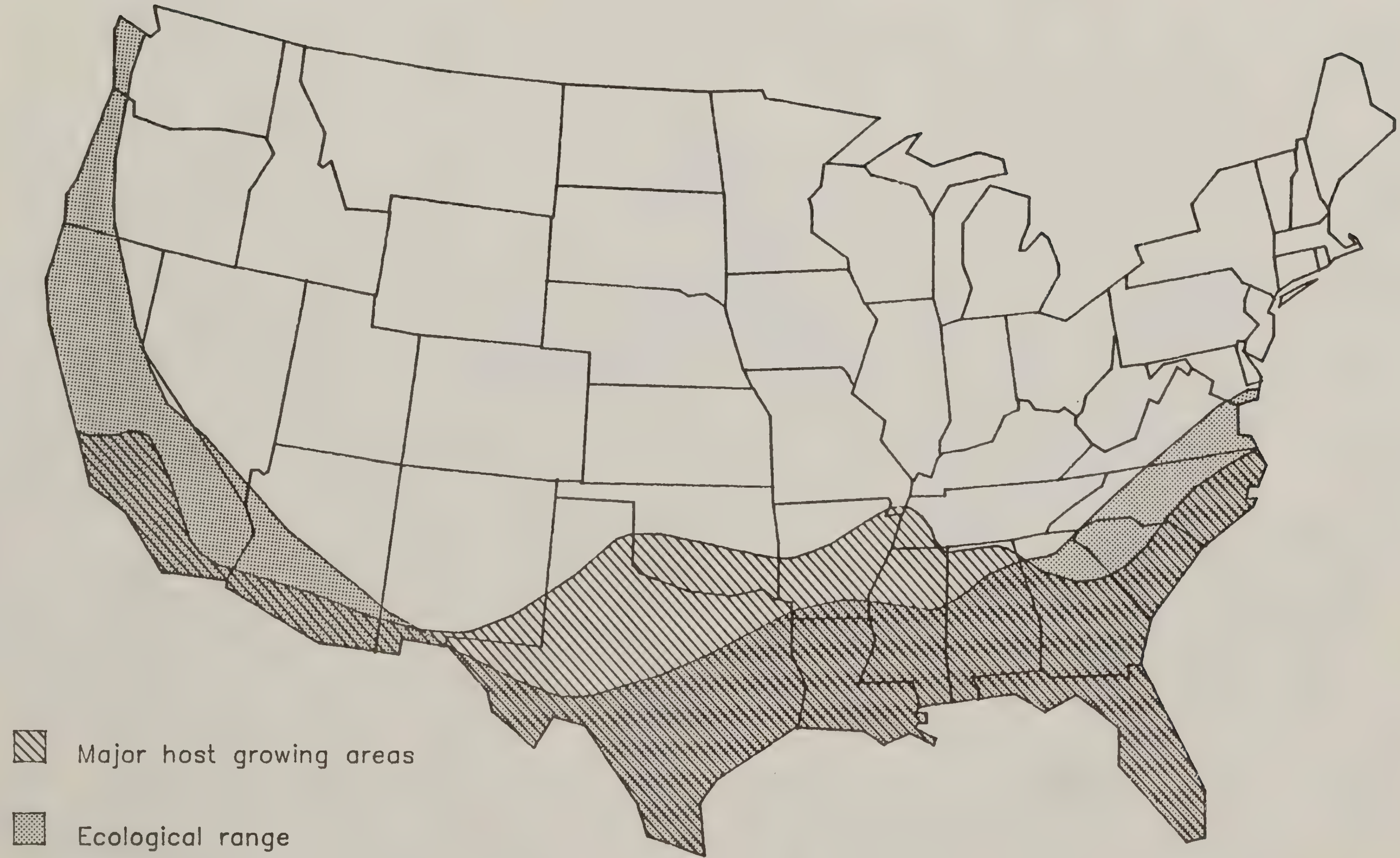
Non-target species that may be captured: Another exotic, *Cryptophlebia* sp. (*C. peltastica*) is attracted to this bait. A noctuid, *Hyperstrotia* sp. is also attracted.

Otis Methods Development Center 11/27/85

Cryptophlebia leucotreta

World distribution

Cryptophlebia leucotreta



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Cydia funebrana (=*Grapholitha*)   Plum fruit moth

Hosts: Plum, Cherry, Apple, Peach, Apricot, Pear, Walnut

Distribution: See map

Biology: This tortricid overwinters as prepupae in cocoons under bark flaps. It has a facultative diapause induced in 2nd and 3rd instar larvae by day lengths less than 14 hours, and completes two generations in temperate areas, but three in S.W. Hungary and Iran. Adults emerge in the spring at 30 accumulated degree-days C (ddC), based on a $10^{o}C$ developmental threshold, with a generation time of 420 ddC. The second generation flight period begins between 450-500 ddC (June-July). Females lay 49-150 eggs singly on leaves or fruit. Larval feeding in fruit causes a characteristic emission of gum, and first generation larvae may cause premature fruit drop. Second generation larvae cause the greatest damage in later fruiting varieties.

Potential U.S. distribution: Throughout the US wherever host plants occur.

Recommended survey areas: Major plum, cherry, apple and peach producing areas (see map). WA, NY, MI, CA, PA, VA, NC, WV, OR, NJ, IL, MA, ME, ID, CO, MD, OH, MO, NH, WI, IN, UT, VT, CT, MT

Pheromone: 95:5 mixture of (Z):(E)-8-dodecenyl acetate
dispenser type - rubber septa
field life - 4 weeks, replace baits midseason or every 4 weeks which ever time period is shorter

Commercial sources of pheromone dispensers: United Agri Products

Traps: United Agri Products, Trece (Wing Trap)

Trap placement: within orchards of host trees, suspended from limbs ca. 1.5m high.

Recommended combinations: Plum fruit moth baits can be combined with no more than one of the following baits: gypsy moth, *Lymantria dispar*, or codling moth, *Laspeyresia* (*Cydia*) *pomonella*.

Combination #1 - Traps baited for *C. funebrana* and *L. dispar* should be located in orchards which are hosts of *C. funebrana* but near potential hosts for *L. dispar*. Plum fruit males are relatively weak dispersers compared to gypsy moth males.

Pheromone dispensers for *L. dispar* should be USDA dispensers (Hercon).

Combination #2 - Traps baited for *C. funebrana* and *L. pomonella* should be placed in orchards which both species use as a host or in mixed orchard situations where favored hosts of both species are available. In

the latter case, the trap should be placed in the orchard which is the favored host (i.e. plum) of C. funebrana because these males are relatively weak dispersers compared to L. pomonella males.

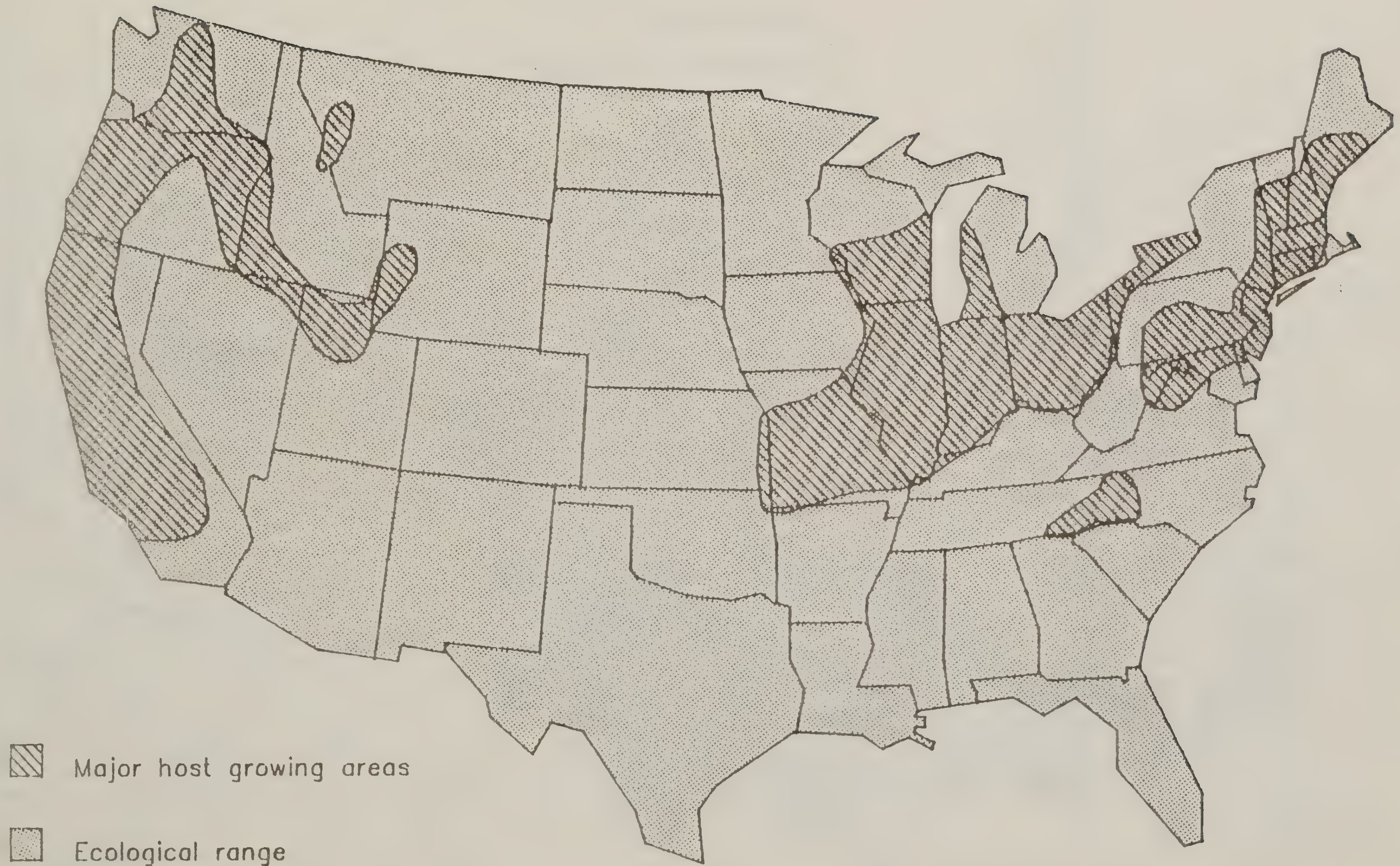
Pheromone dispensers for L. pomonella available from United Agri Products.

Non-target species that may be captured: Grapholita prunivera, G. molesta, Phyllonorycter blancardella (Lepidoptera:Gracillariidae)

Otis Methods Development Center 11/27/85

Cydia funebrana
World distribution

Cydia funebrana



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Epiphyas postvittana Light brown apple moth

Hosts: This species has a wide host range, and has been reported to feed on at least 73 plant species from 27 families. Economic damage, however, occurs most commonly to apples.

Distribution: See map.

Biology: In southern Australia and New Zealand this tortricid has three generations per year and overwinters as a larva. All stages have a lower threshold for development of 7.5°C and, with no mention of diapause in the literature, this species would most likely be limited to the southern U.S.

Female moths deposit egg masses on the upper leaf surface or on fruit. After dispersing, newly hatched larvae construct silken shelters on the underside of leaves, usually near a midrib or large vein. Older larvae roll together leaves and buds or fruit with webbing. Larvae feed and then pupate within these "nests".

Potential U.S. Distribution: Where the average minimal temperatures are above -10°C (see map).

Recommended Survey Areas: Major apple producing states within ecological range (see map) CA, OR, NC.

Pheromone: 25:1 mixture of (E)-11-Tetradecen-1-ol acetate:
(E,E)-9, 11-Tetradecadien-1-ol acetate.
Loading rate - 1.0 mg
Dispenser type - Rubber Septa
Field Life - 30 days - replace baits every 30 days.

Source of Pheromone Dispensers: Otis Methods Development Center.

Traps: United Agri Products and Trece Wing Traps

Trap Placement: Within orchards of host (apple, pear). Suspend traps from the limbs of trees ca 1.5m in height.

Recommended Combinations: None presently recommended.

Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic non-targets is unknown.

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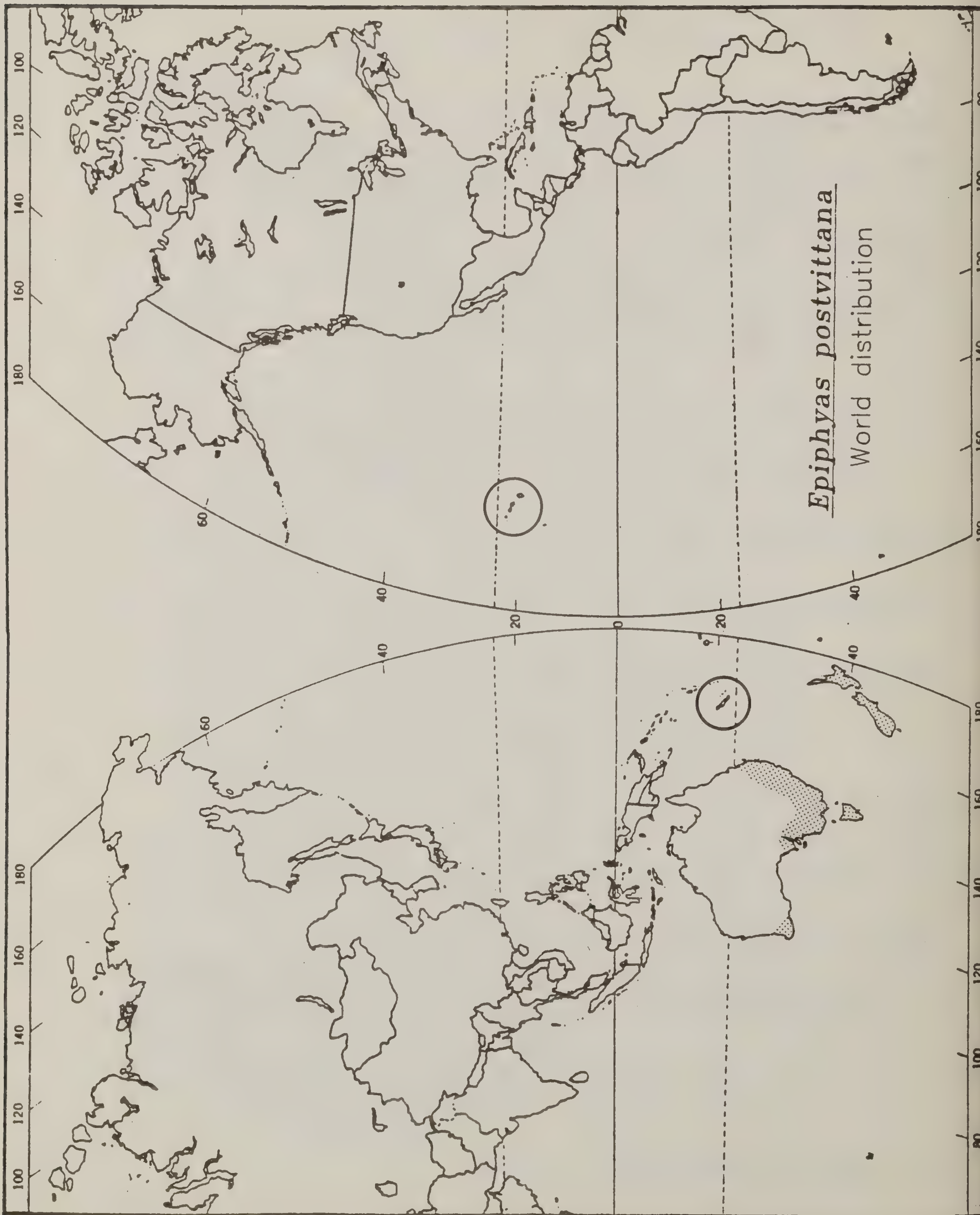
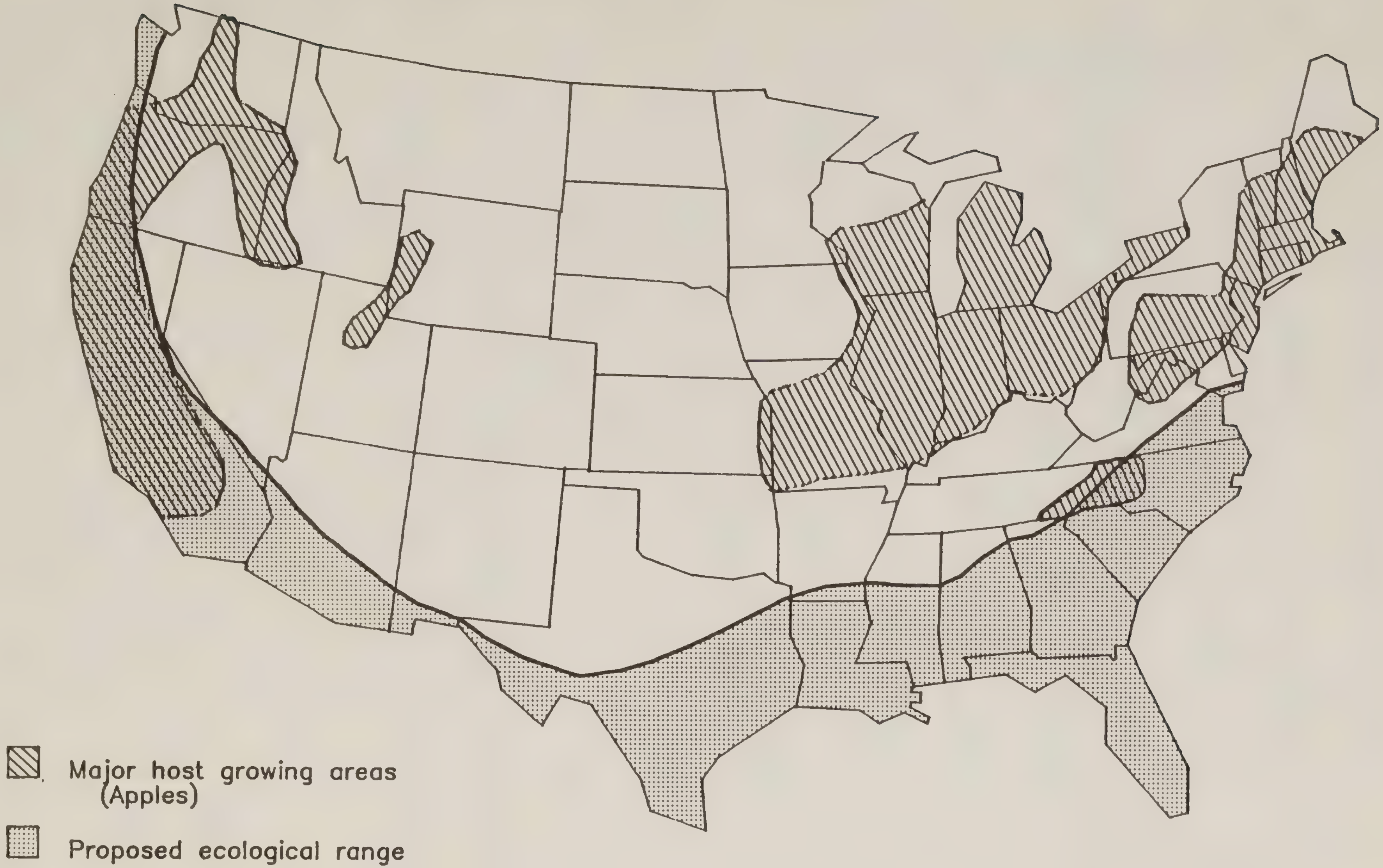


Epiphyas postvittana
World distribution

Epiphyas postvittana



Eupoecilia (=Clysia) ambiguella European grape berry moth

Hosts: Grapes

Distribution: See map

Biology: Throughout Europe, there are two generations per year with the pupa as the overwintering stage. In Bulgaria, adults emerge in May and second generation adults occur around the first of July. First generation eggs are laid on flower buds, whereas second generation eggs are laid on grapes. Larvae feed on the flowers or grapes then pupate, usually under bark flaps.

Potential U.S. Distribution: Throughout the country wherever host plants occur.

Recommended Survey Area: Major grape producing states (see map). CA, NY, WA, MI, PA, OH, AZ, AR, NC, MO.

Pheromone: 5:1 mixture of Dodecen-1-ol acetate:(Z)-9-Dodecen-1-ol acetate
loading rate - 6.0mg
dispenser type - rubber septa or plastic laminate
field life - 42 days - replace baits every 42 days.

Source of Pheromone Dispensers: Otis Methods Development Center; Hercon Corp.; Trece, United Agri Products

Traps: Trece and United Agri Products Wing Trap

Trap Placement: Within grape vineyards: ca 0.5-1.0m in height.

Recommended Combinations: Eupocilia ambiguella baits can be combined with those for Lobesia botrana within a single trap.

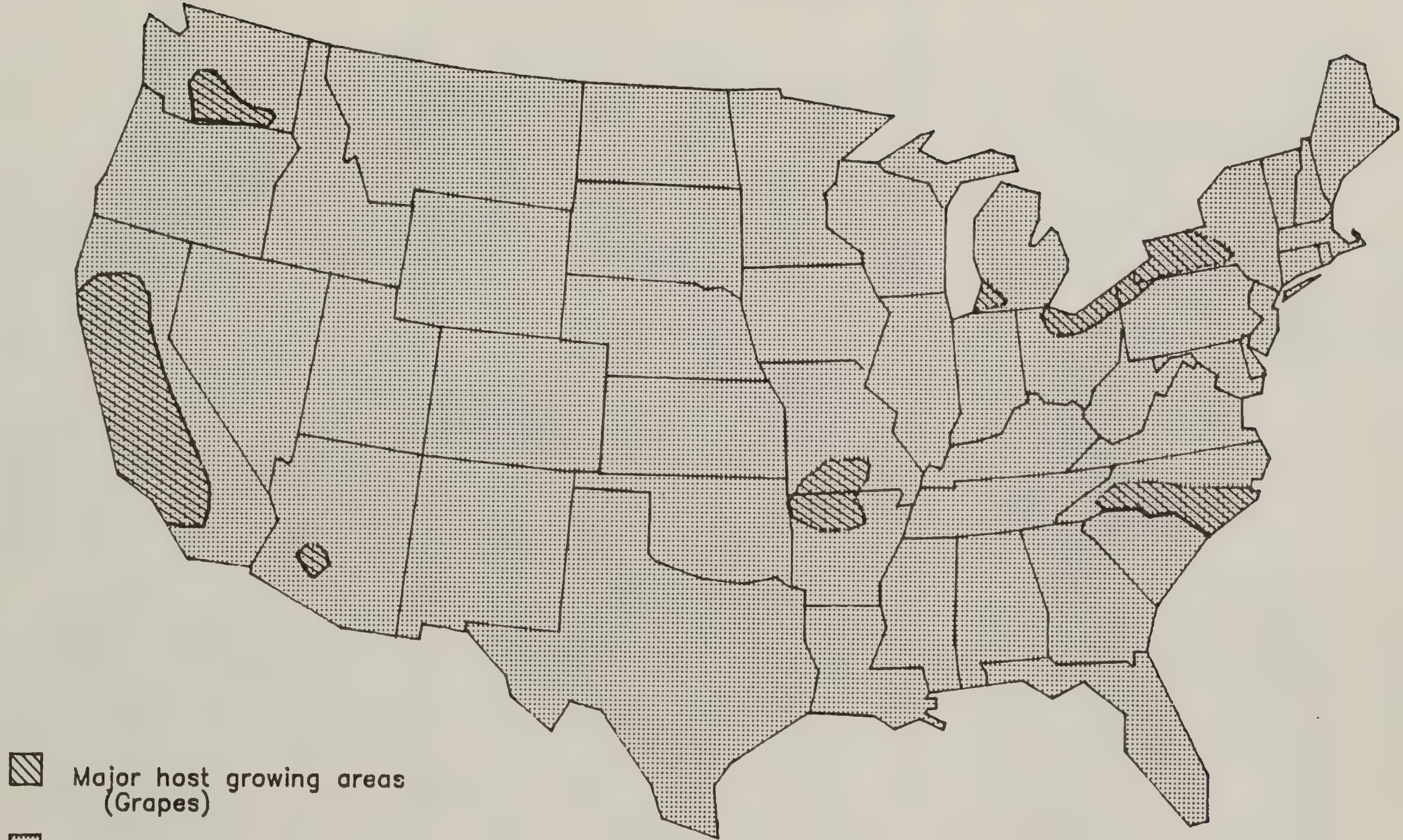
Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic no -targets is unknown.

Otis Methods Development Center - 11/27/85

Eupoecilia ambiguella

World distribution

Eupoecilia ambiguella



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Lobesia botrana Grape vine moth

Hosts: Grapes (Vitis)

Distribution: See map

Biology: *Lobesia botrana* is a multivoltine species with four generations per year, depending on latitude. Diapause is facultative, occurring in the pupal stage whenever the eggs or early larval stages are exposed to day-lengths of less than 12 hrs. Over-wintering pupae live within cocoons located under fallen leaves or in cracks in the soil or under the grape vine bark.

Spring adult emergence will begin whenever the daily average air temperature is above the minimal threshold temperature of 10°C for 10-12 days. Traps for monitoring spring adult flight should be set up after 60 degree-days C (ddC). Adults will fly at dusk whenever the temperature is above 12°C, but rainfall or wind will reduce flight.

First generation eggs are laid on the flower buds or pedicels of the vine. The larvae feed on the bud clusters before pupating inside them or under the rolled leaf . It takes an average of 402 ddC to complete the first generation from sexual maturation of the parents to pupation.

The second generation eggs are laid singly on individual grapes. The larva will enter the grape and feed before pupating inside the grape. To complete the second generation, 441 ddC are required.

The third generation larvae also feed on the grapes but, unlike the second generation, will feed on more than one grape. The third generation normally produces diapausing pupae but may also give rise to a partial fourth generation.

Potential U.S. distribution: Throughout the U.S., wherever host plants occur (see map).

Recommended survey area: Major grape producing States (see map). CA, NY, WA, MI, PA, OH, AZ, AR, NC, MO

Pheromone: (E,Z)-7,9-dodecenyl acetate
dispenser type - rubber septa
field life - 3 weeks, replace baits every 3 weeks.

Commercial source of pheromone dispensers: Trece Corp., United Agri Products

Traps: Trece, United Agri Products (Wing Trap).

Trap placement: *Lobesia botrana* males are weak dispersers; therefore traps should be placed within grape vineyards. Trap should be suspended from wires or vines ca. 1/2 to 1 m above the ground. Care should be exercised in trap placement so that grape foliage does not block trap entry ports.

Recommended combinations: Compatible pheromones include attractants for the gypsy moth, *Lymantria dispar*, the codling moth, *Laspeyresia* (*Cydia*) *pomonella* and the European grape berry moth, *Eupoecilia* (*Clysia*) *ambiguella*. Only one of these attractants should be combined at a time in traps baited for *Lobesia botrana*.

Combination #1 — Traps baited for *L. botrana* and *L. dispar* should be placed in vineyards located close (within 300 m) to hosts for the gypsy moth.

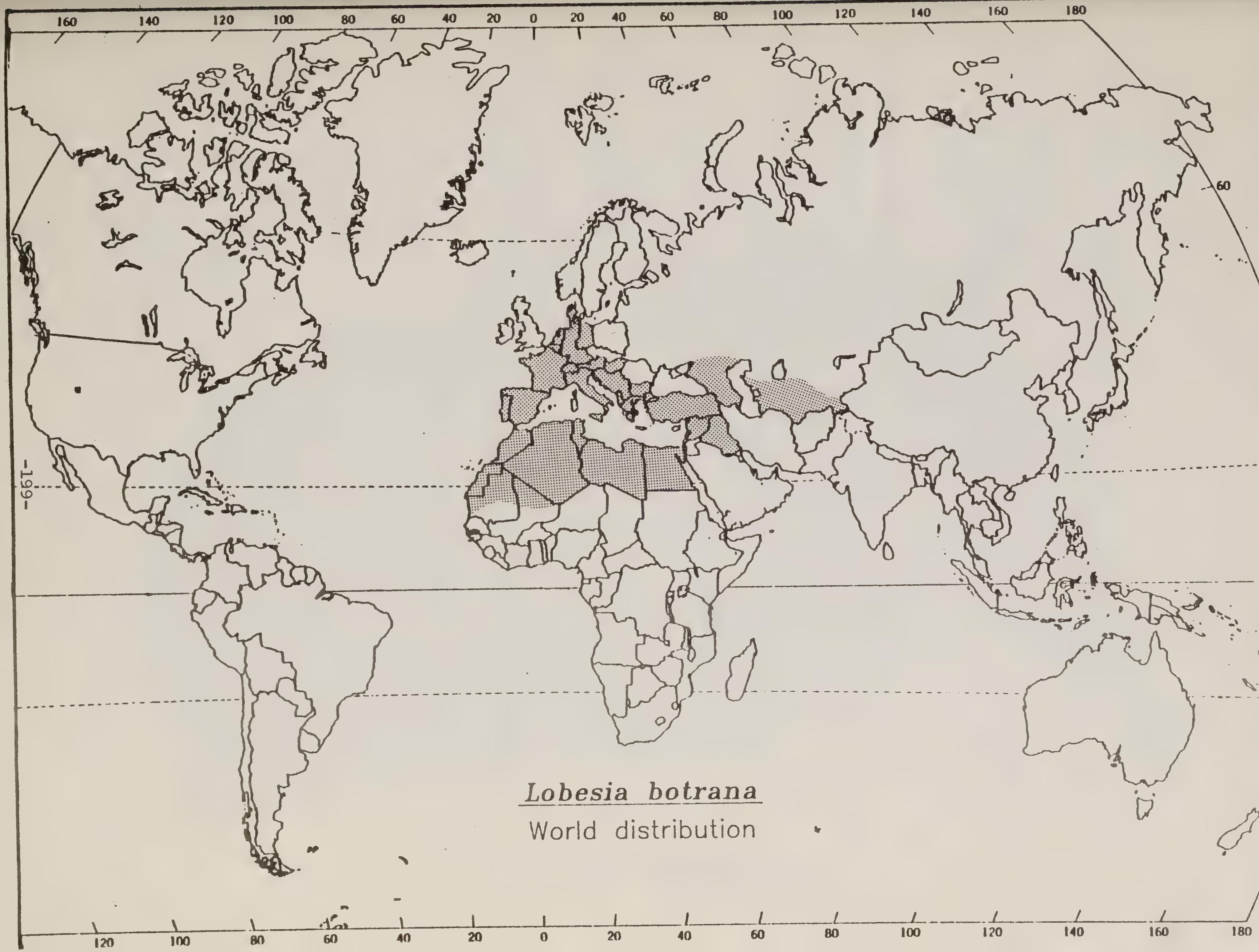
Pheromone dispensers for *L. dispar* should be USDA dispensers (Hercon).

Combination #2 — Traps baited for *L. botrana* and *L. pomonella* should be placed in vineyards located adjacent to host (apple, pear, etc.) for the codling moth. In considering this combination, the effect of monitoring codling moth population with traps placed outside of the host crop will have to be weighed against the objective of the monitoring program (i.e. timing spray application, etc.).

Pheromone dispensers for *L. pomonella* available from United Agri Products.

Combination #3 — Traps baited for *L. botrana* and *E. ambiguella* (both exotics) placed in vineyards. See recommendations for *E. ambiguella* for more trap placement information.

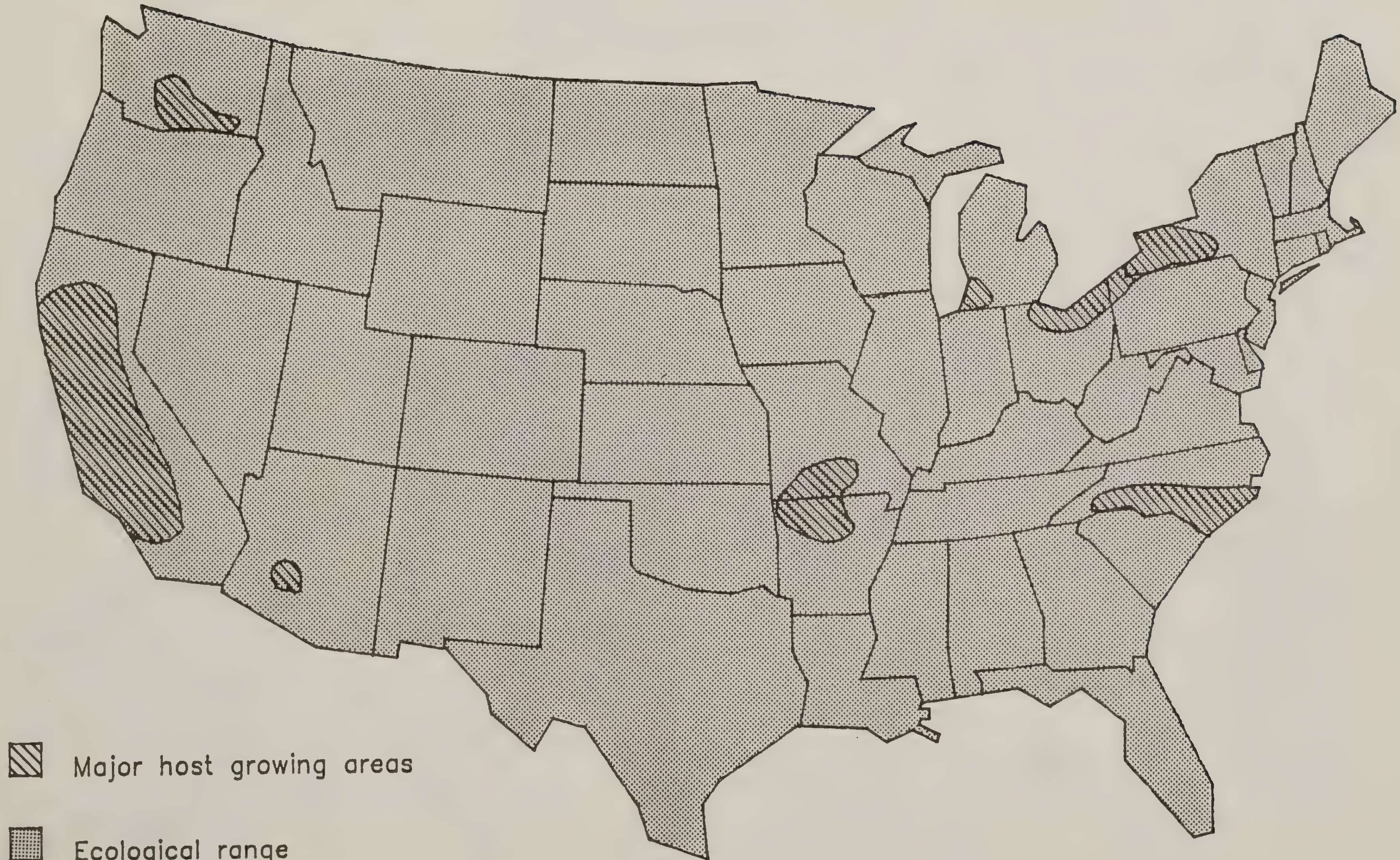
Non-target species that may be captured: No reports of major trap-loading by domestic non-target species have been noted.



Lobesia botrana

World distribution

Lobesia botrana



Mamestra brassicae Cabbage moth

Hosts: Larvae of the cabbage moth are general feeders on many vegetables and field crops but damage is most often reported on crucifers.

Distribution: See map

Biology: Eggs are deposited in masses on the underside of leaves. Larvae feed on the leaves and in some instances, i.e. cabbage, bore into the head or stalk. Pupation occurs in the soil. There can be from one to two generations per year depending on climate. Overwintering occurs in the pupal stage.

Potential U.S. Distribution: Throughout the U.S.

Recommended Survey Areas: Major crucifer producing states (see map). CA, TX, NY, OR, AZ, MI.

Pheromone: Z-11-Hexadecen-1-ol acetate
loading rate - 1 mg.
dispenser type - poly caps or rubber septa
field life - 90 days

Source of Pheromone Dispensers: Otis Methods Development Center

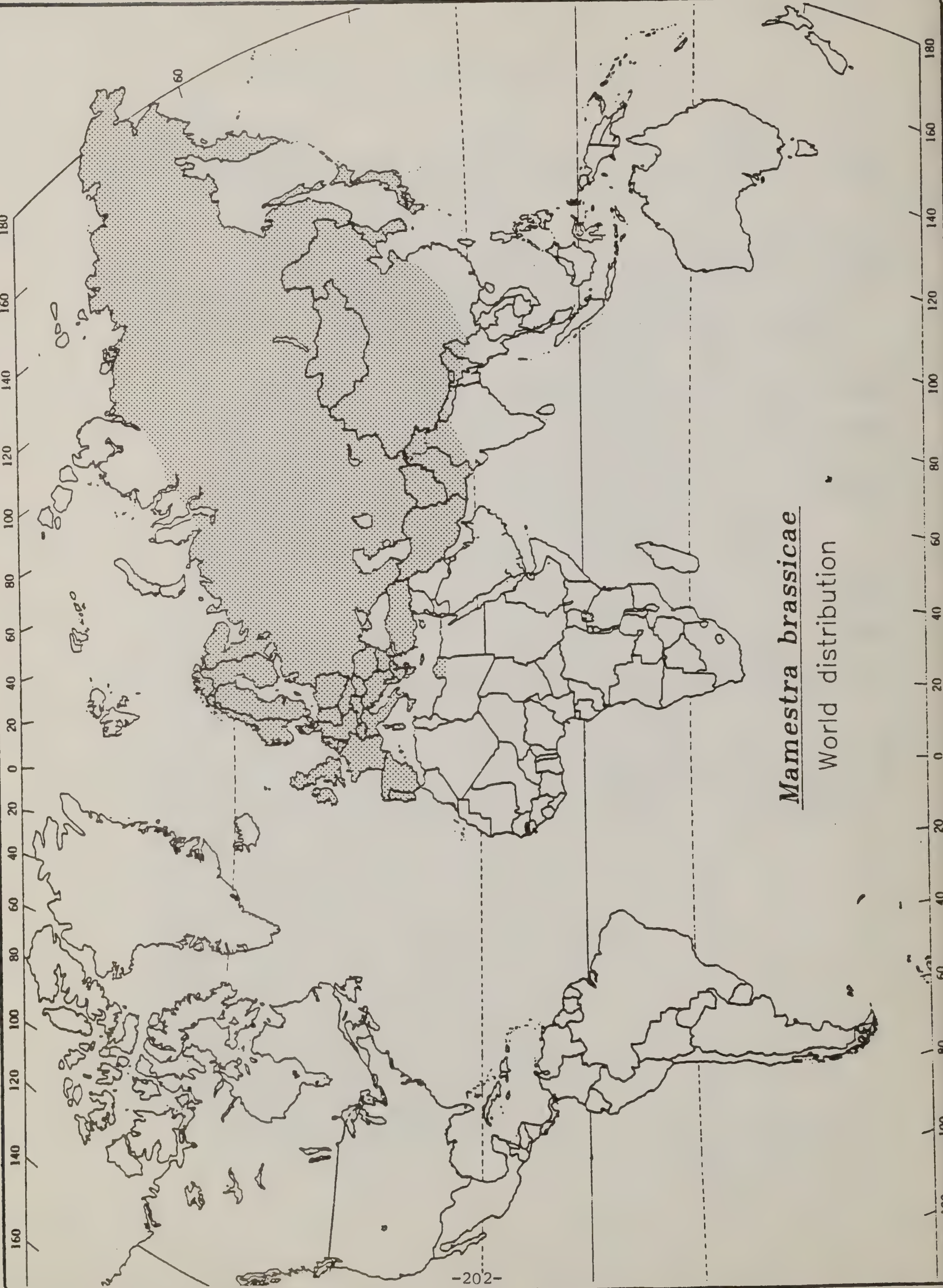
Traps: Trece and United Agri Products Wing Trap

Trap Placement: Within fields of host crops; trap should be placed on stakes at approximately the crop height and raised as the crop matures.

Recommended Combinations: None presently recommended.

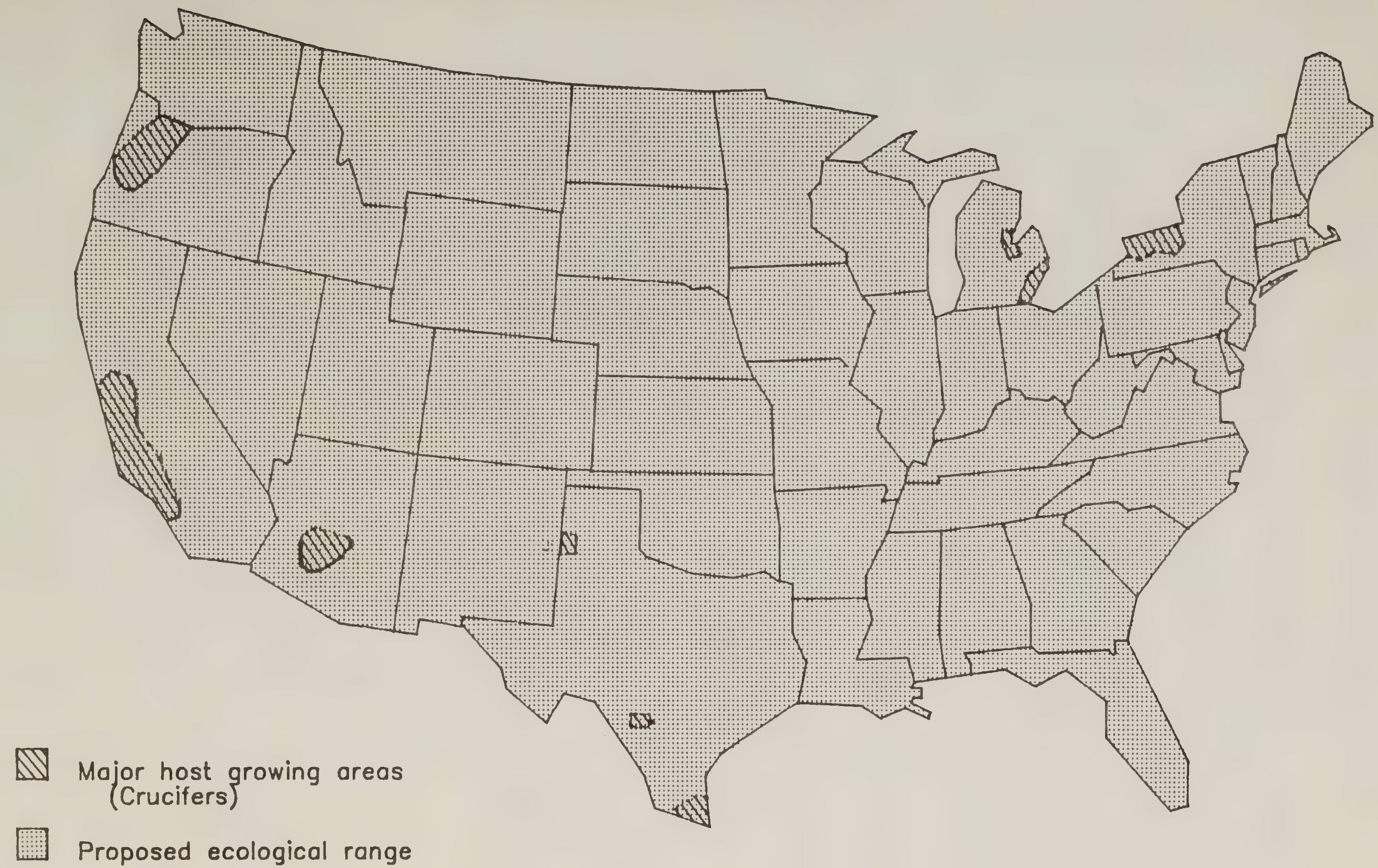
Non-target species that may be captured: Results of 1985 pilot trapping are incomplete, therefore risk of trap loading by domestic non-targets is unknown.

Otis Methods Devevlopment Center - 11/27/85



Mamestra brassicae
World distribution

Mamestra brassicae



<u>Rhagoletis</u> <u>cerasi</u>   European cherry fruit fly

<u>Hosts</u>: Cherry, <u>Lonicera</u>

<u>Distribution</u>: See map

<u>Biology</u>: This fruit fly has one generation per year and overwinters as a puparium in the soil. In Switzerland, adult emergence occurs in the spring, after 430 degree-days C have accumulated at a soil depth of 5 cm, based on a 5°C developmental threshold,. This usually occurs in May or June, with the flight period lasting from one to two months. Puparia require cold soil temperatures (less than 0°C), for at least one month, for the majority to break diapause. Eggs are laid in the fruit where the larvae feed for 13-30 days. Damage can be as severe as in Italy, where up to 90 percent of the fruit has been infested.

<u>Potential U.S. distribution</u>: Throughout the U.S., wherever host plants occur (see map).

<u>Recommended survey area</u>: Major cherry producing States (see map). MI, WA, OR, CA, NY, PA, UT, WI, MT, CO, ID

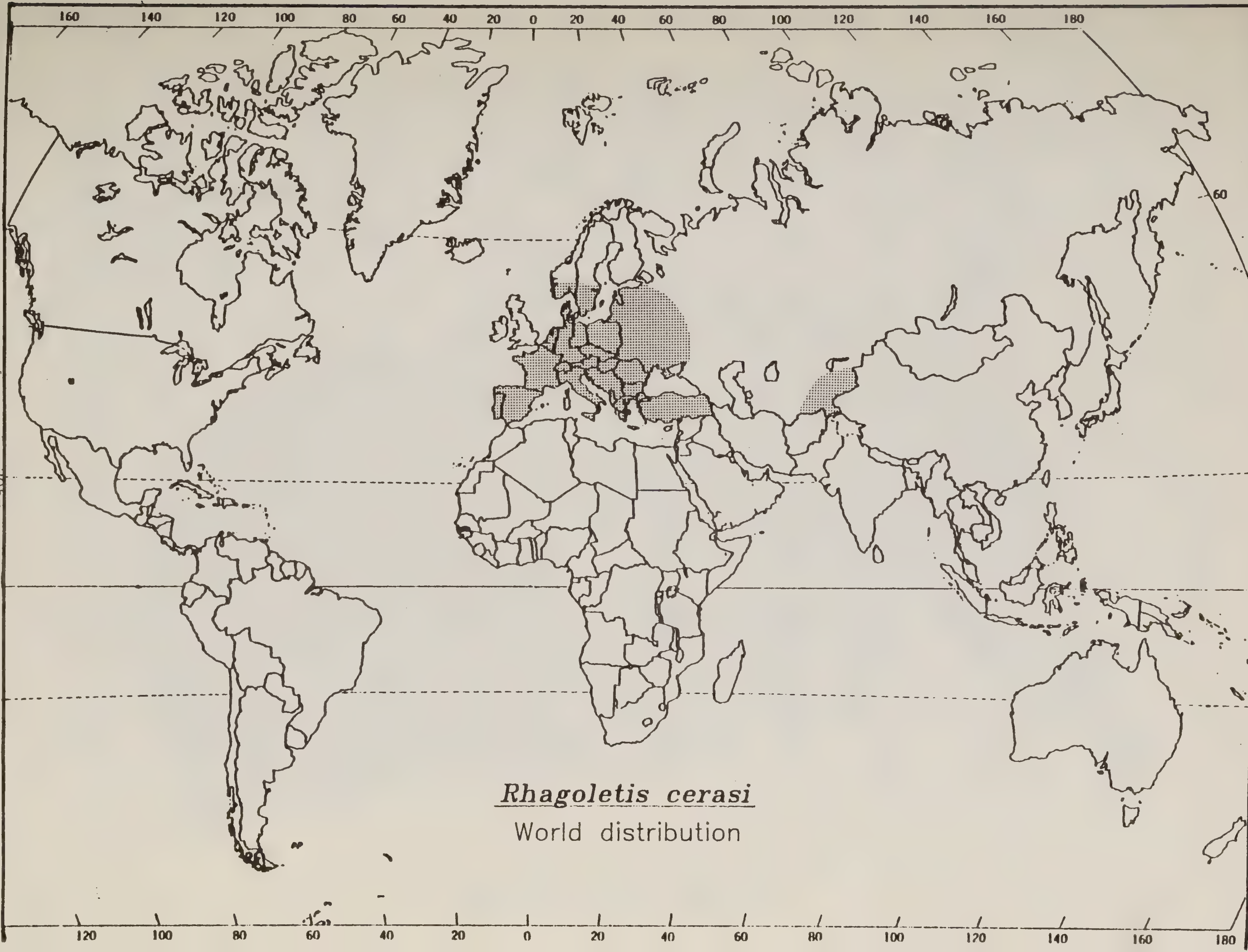
<u>Attractant</u>: Ammonium acetate or Ammonium carbonate

<u>Commercial sources of attractant dispensers</u>: Trece Corp.

<u>Traps</u>: Colored sticky panels from Great Lakes IPM Corporation; Swiss Federal Research Station

<u>Recommended combinations</u>: Because the attractants (visual and olfactory) employed by this trap are used by a variety of fruit pests, monitoring of native species distribution and abundance is also possible.

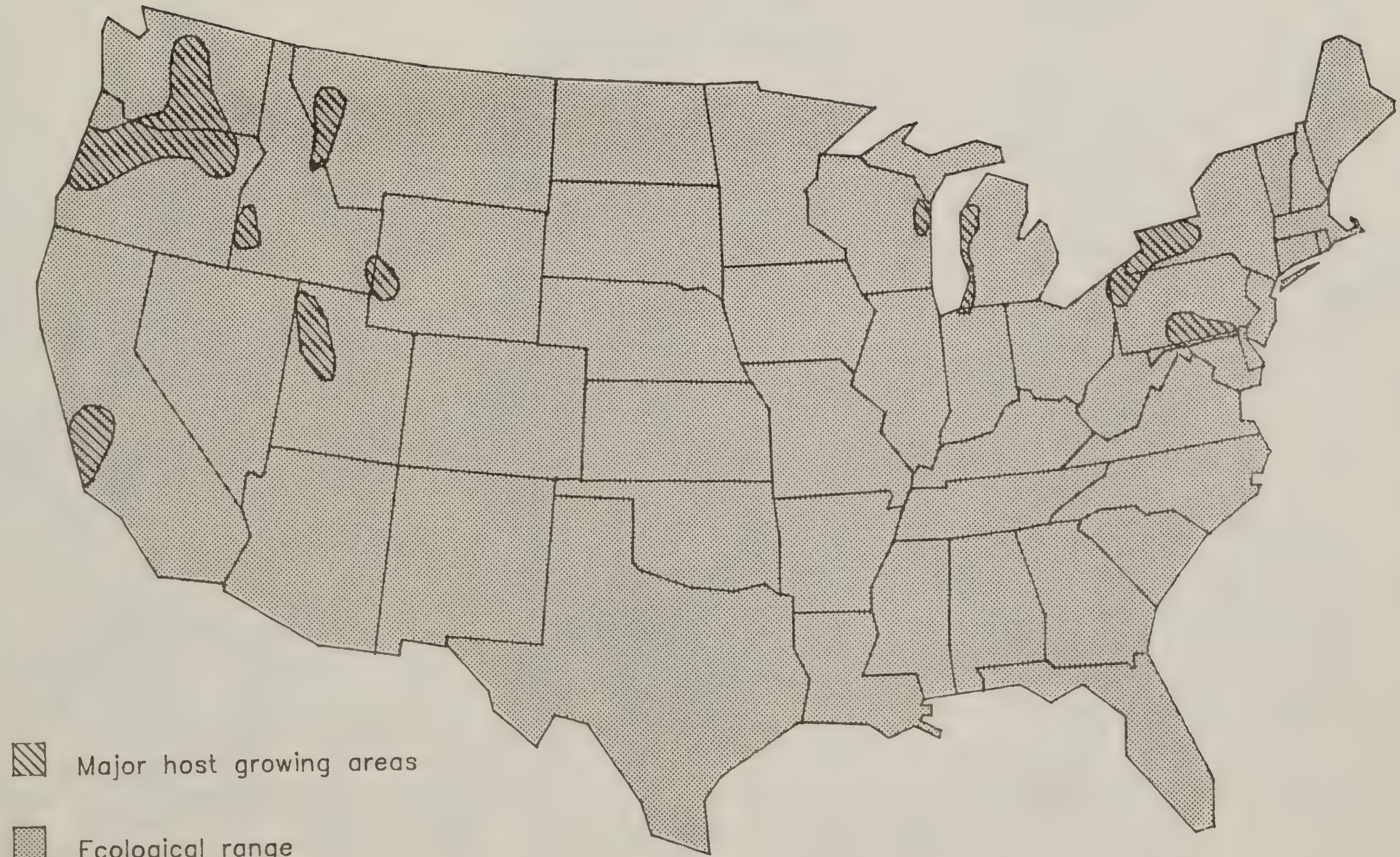
Otis Methods Development Center   11/27/85



Rhagoletis cerasi

World distribution

Rhagoletis cerasi



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Spodoptera littoralis   Egyptian cottonworm (Egyptian cotton leafworm)

Hosts: Cotton, tobacco, alfalfa, soybeans, clover, vegetables

Distribution: See map

Biology: Spodoptera littoralis is a multivoltine species that does not enter a diapause stage, nor can it tolerate long periods of temperatures at 13°C or lower. S. littoralis can over-winter in southern Spain, but not in northern Italy or France.

The eggs are laid on the leaves of host plants and begin to hatch after 28.6 degree-days C (ddC) at a base temperature of 14.8°C. The optimal temperature for hatch is 28-30°C. Exposing the eggs to 13°C for eighteen days will result in complete egg mortality.

Newly-emerged larvae will feed on the leaves of cotton, but not on the large veins. Later instar larvae disperse widely, become nocturnal in habit, and will at times attack the young buds and cotton bolls. The larvae weaken the cotton plants and leave the plants susceptible to damage by the bollworms.

The optimal temperature for larval development is 25°C, and at a base temperature of 13°C, 257.1 ddC are required to complete the larval stage. Exposing the larvae constantly to 13°C does not prevent the larvae from forming prepupae, but all the prepupae will die.

Larvae pupate in the soil, and at the 13°C base temperature, male and female pupae complete their development in 177.1 and 153.5 ddC, respectively. Exposing the pupae to 13°C for seventy days will result in few adults emerging, and those that do emerge will be deformed and incapable of mating. Exposing the pupae to temperatures above 30°C will also result in poor survival. The females which do emerge will deposit many non-viable eggs. The optimal temperature for pupal survival is 20°C.

The adults emerge at night, with the males emerging about three hours after the females. The males can mate 5-6 times, but usually mate only once a night. The females will mate, at the most, twice. Few males will fly at temperatures below 13°C. The distance the adults migrate is unknown, although marked moths have been captured as far as 1500 meters from a release site. An infestation in France is thought to have come from migration of adults from overwintering areas in southern Spain.

Potential U.S. distribution: Where the average annual minimal temperatures are above 10°C (see map).

Recommended survey area: Major cotton producing states (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL.

Pheromone: 99.5:0.5 mixture of (Z,E)-9,11:(Z,E)-9,12-tetradecadienyl acetates
dispenser type - rubber septa or poly cap or plastic laminate
field life - 2 weeks, replace lure dispensers every 2 weeks.

Commercial source of pheromone dispensers: Hercon, Trece

Traps: Trece, United Agri Products (Wing Trap)

Trap placement: Traps should be hung from stakes at approximately the height of the crop. As the season progresses, the trap should be raised as the crop height increases.

Recommended combinations: Egyptian cottonworm *S. littoralis* baits can be combined in traps with baits for the following exotic pests: rice cutworm *Spodoptera litura*, *Heliothis armigera* and *Pectinophora scutigera*. *S. littoralis* can also be included in domestic survey for *P. gossypiella*.

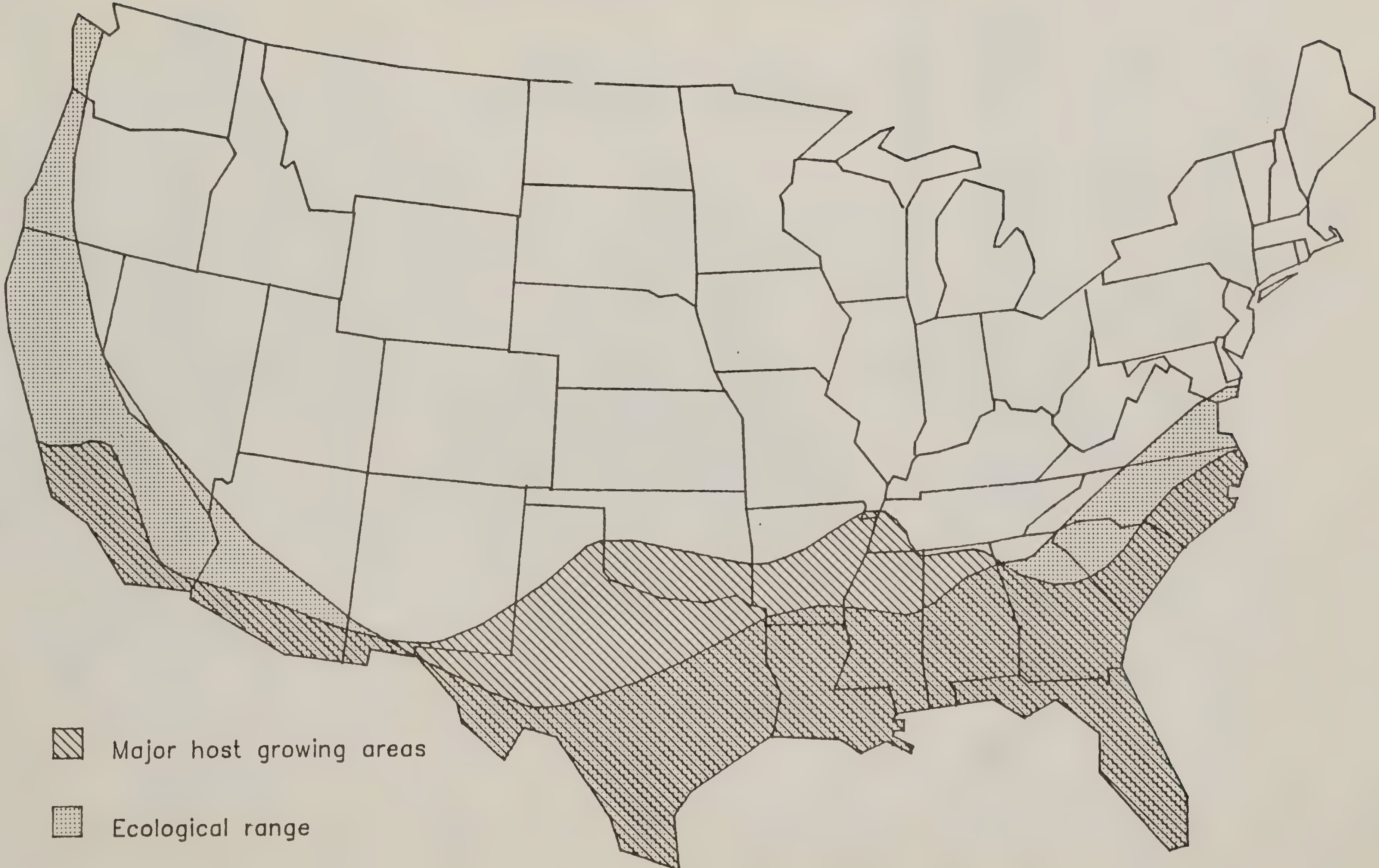
Non-target species that may be captured: The noctuid *Erastria* sp. has been reported in *S. littoralis* traps.

Otis Methods Development Center 11/27/85

Spodoptera littoralis

World distribution

Spodoptera littoralis



Spodoptera litura Rice cutworm (cotton leafworm)

Hosts: Cotton, Tobacco, Grapes, Corn, Soybeans, Vegetables

Distribution: See map

Biology: *Spodoptera litura* is a multivoltine species with no known diapause stage. It has 2 generations/year in China, 4 to 5 generations/year in Japan and up to 8 generations/year in Taiwan. Temperatures of 10°C or lower will cause mortality in all the life stages with the most cold resistent stages capable of surviving -2°C for only 1 day.

A generation normally requires 526.3 degree-days C at a base temperature of 10.3°C. The eggs hatch in 4 days at 26.7°C. Newly hatched larvae are very susceptible to dry heat; consequently, they usually stay on the lower leaf surfaces during the day and feed at night. During the last two instars, the larvae feed only at night and find shelter during the day under the lowest leaves or in the soil at the base of the host plants. The larvae either defoliate the plant or cut it off like a cutworm.

At 28.6°C larvae pass through 6 instars in approximately 13 days and pupate within earthen cells. The pupal stage is completed in 7.3 and 6.1 days for male and female pupae, respectively, at 28.6°C.

The adults emerge at night between 11:00 p.m. and 3:00 a.m. The males can fly up to 5 km/night; however, flight is greatly reduced at temperatures below 20°C. The males will mate once each night and will avoid any females mated previously.

The females begin to deposit their eggs 2 to 3 days after emerging. The eggs are deposited at night in batches of up to 300 eggs on the under-surface of host leaves. A female can deposit from 6 to 9 batches of eggs over a 7 day life span.

Potential U.S. distribution: Where the average annual minimal temperatures are above 10°C (see map).

Recommended survey area: Major cotton producing States, and Florida (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL.

Pheromone: 88:12 mixture (Z,E)-9,11:(Z,E)-9,12 tetradecadienyl acetate
dispenser type - rubber septa or polycaps
field life - 2 weeks, replace lure dispensers every 2 weeks

Commercial sources of pheromone dispensers: Trece and United Agri Products

Traps: Trece, United Agri Products (Wing Trap)

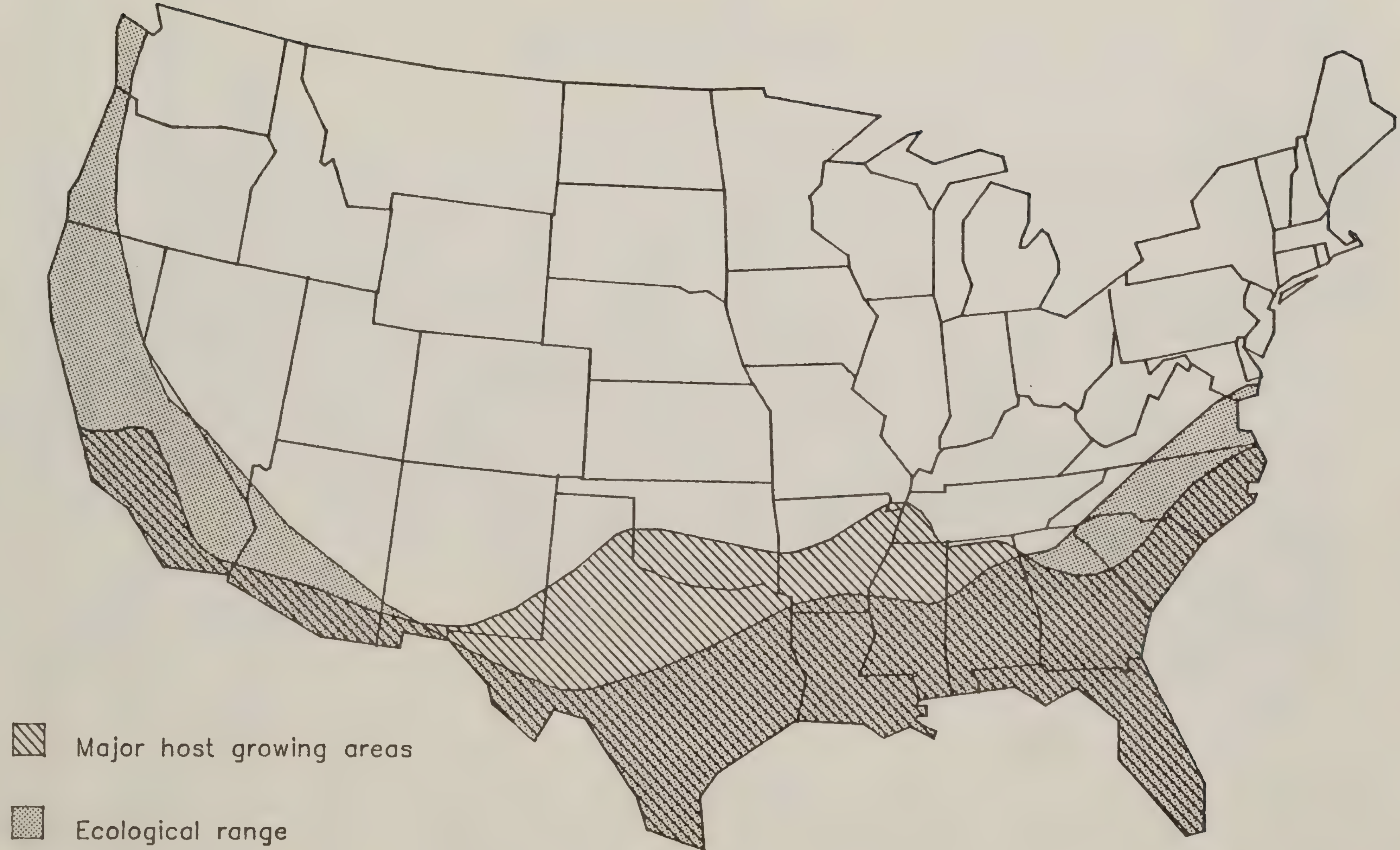
Trap placement: Trap should be hung from stakes at approximately the height of the crop. As the season progresses, the trap should be raised as the crop height increases.

Recommended combinations: Rice cutworm (*S. litura*) baits can be combined in traps with baits for the Egyptian cottonworm *Spodoptera littoralis*. Traps baited for *S. litura* and *S. littoralis* can be placed in any of the following crops: cotton, tobacco, soybeans, alfalfa, clover or vegetables.

Spodoptera litura

World distribution

<u>*Spodoptera litura*</u>



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Plant Protection
and Quarantine

1985 Pest Recognition Sheets

Exotic Pheromone Trapping Project

Note:
Pinned specimens of suspected exotics from this exotic trapping project may be sent to:

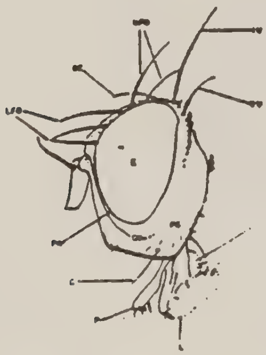
Systematic Entomology Laboratory
Room 01, Building 003
BARC-West
Beltsville, MD 20705

Please submit specimens with a PPQ Form 391 and designate as urgent material from APHIS-PPQ EXOTIC TRAPPING PROJECT.

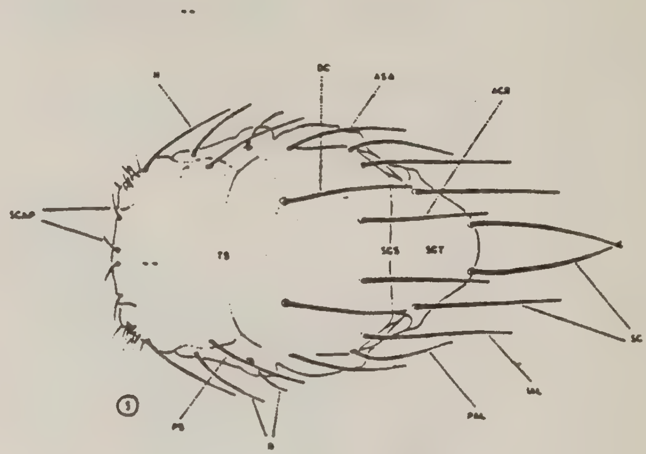
Cooperative National Plant Pest Survey
and Detection Program

Rhagoletis cerasi (Linnaeus) (DIPTERA: TEPHRITIDAE)

Distribution: Europe



head, lateral view

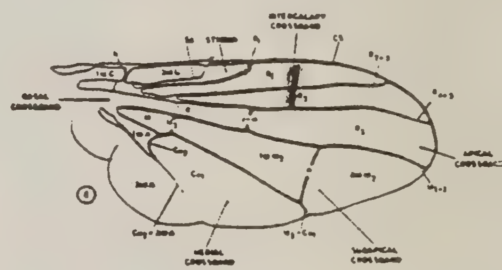


thorax, dorsal view

The genus Rhagoletis has the following characters:

1. An ivory to yellowish-white stripe reaching from the humeral callus (shoulder) to the base of the wings.

2. A wing pattern of transverse yellowish to brownish black bands; with r-m crossvein at the center of the first M2 cell.



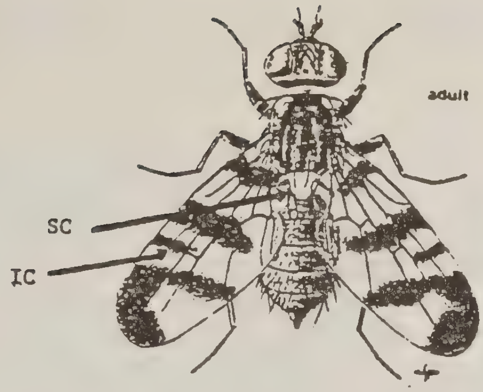
wing venation

3. The frons is slightly wider at the vertex than at the level of the antennae, but is narrower than the maximum width of the eye; gena (GN) about 0.12 to 0.23 height of head.

4. Ocellar bristles (OC) on head approximately same length as upper fronto-orbital bristles (UFO); three pairs of convergent lower fronto-orbitals (LFO) between eyes, two pairs of reclinate divergent upper fronto-orbitals (UFO),

5. Dorsocentral bristles (DC) of thorax located slightly before, on, or slightly behind a line drawn between anterior supraalar bristles (ASA). They are always closer to supraalars than to either transverse sulcus or to acrostichal bristles (ACR).

6. Femora of second and third pair of legs without well-developed spines along bottom of margin.



adult, Rhagoletis cerasi (L.)

Rhagoletis cerasi (L.) can be separated from other species of Rhagoletis in North America by the following characters:

1. The scutellum (SC) is completely cream to yellowish-white without a distinct spot; at base only, it may be dark on the sides.

2. The wing pattern has a small intercalary crossband (IC).

3. Body mostly black with yellow to white markings.

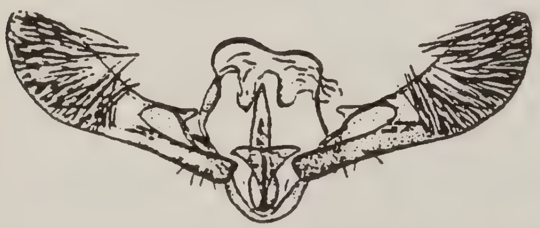
R. alternata (Fallen) (Europe)

R. alternata (Fallen) in Europe resembles R. cerasi but alternata has a slightly different pattern of crossbands.

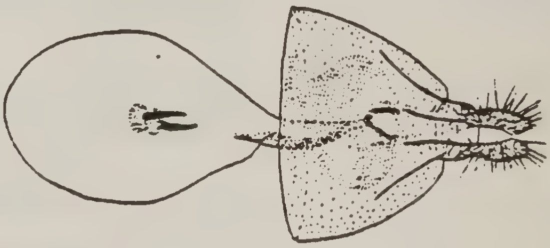
Cryptophlebia leucotreta (Meyrick) (LEPIDOPTERA: TORTRICIDAE)

Distribution: southern Africa

The adults are sexually dimorphic, the male having a wing span of 15-16mm and the famale 19-20mm; in both sexes, the forewing pattern consists of a mixture of plumbeious, brown, black, and ferruginous markings, the most conspicuous being the blackish triangular pretornal marking, and the crescent-shaped marking above it, and a minute white spot in the discal area. Dark markings in the apical portion of the forewings are also typical. The male is at once distinguished from all other species by its specialized hindwing, which is slightly reduced and has a circular pocket of fine hair-like black scales overlaid with broad weakly shining whitish scales in the anal angle, and its heavily tufted hind tibia.



male genitalia



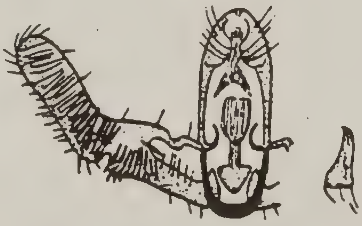
female genitalia

Other genera with expanded tufts on hind legs are Melissopus (one U.S. species), Phaecasiophora (three U.S. species), Cydia injectiva (Heinrich), and some species of Ecdytolopha.

Lobesia botrana (Den. & Schiff.) (LEPIDOPTERA: TORTRICIDAE)

Distribution: Europe, Mediterranean

Wing expanse is 10-17 mm. Male has hindwing white, weakly scaled, female has hindwing dark greyish fuscous. Forewing pattern: the plumbeous suffusion of the ground color of the forewing, forming a subquadrate patch medio-dorsally and bordering the outer edge of the median fascia costally and enclosing the tornal marking, is characteristic of the species.



male genitalia

Resembles Endopiza viteana Clemens (Paralobesia viteana (Clemens)), the grape berry moth, in the United States very closely; but male genitalia are distinct and venation is distinct, i.e., R2 and R3 of forewing rather close together at base in L. botrana, while in E. viteana R2 and R3 are well separated.

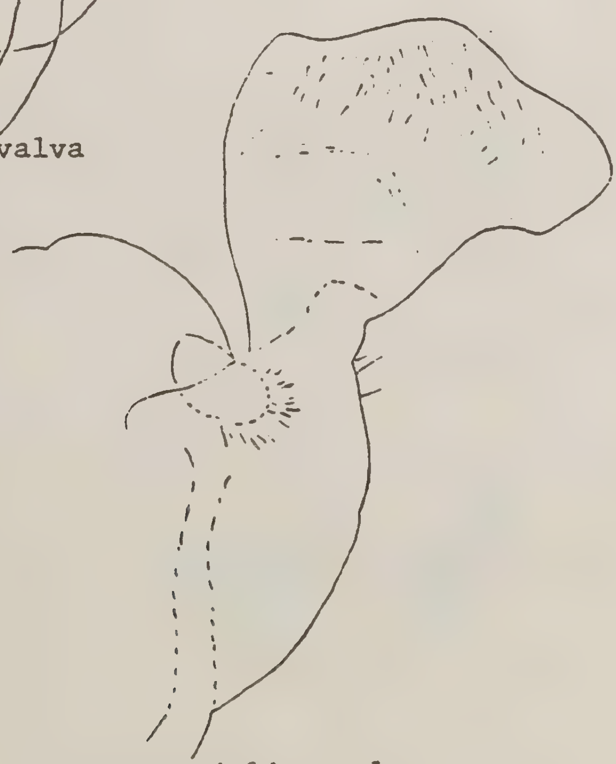
Mamestra brassicae Linnaeus (LEPIDOPTERA: NOCTUIDAE)

DISTRIBUTION: Europe, Asia

The subfamily Hadeninae is recognized by the hair on the surface of the eyes. M. brassicae has the subterminal line not defined by whitish on the inner side, this separates brassicae from M. configurata Walker, a U.S. species which has subterminal line prominently defined by whitish on the inner side; wing expanse of M. brassicae is approximately 44 mm. Differences in male genitalia allow separation of M. brassicae from M. configurata and M. curialis (Sm.). (See figures.)

M. brassicae, valva

M. configurata, valva



M. curialis, valva

Epiphyas postvittana (Walker) (LEPIDOPTERA: TORTRICIDAE)

DISTRIBUTION: Australia, New Zealand, Hawaii, England

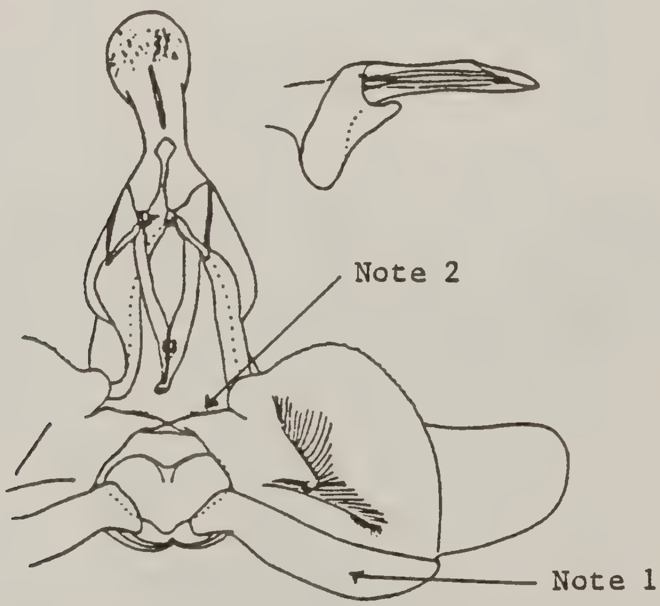
Sexual dimorphism pronounced. MALE: forewing expanse 16-21 mm; basal half of forewing light buff or pale yellow, sharply contrasting with the dark brown and brownish-red coloration of distal half, pre-apical spot on costa obscure; hindwing grey. The wing pattern is extremely variable. There are lightly marked forms which resemble the female in which there is only an oblique median fascia and a pre-apical spot noticeable, and there are forms in which the distal half is extremely dark, varying from reddish-brown to blackish often with purplish mottling, the contrasting pale basal half may be sparsely speckled with black. FEMALE: forewing expanse 17-25 mm., apex more produced than male; coloration of forewing more uniform with less contrast between basal and distal halves, pre-apical spot on costa more noticeable, oblique median fascia usually reduced.

Epiphyas: form of valva with its basal processes and sacculus extending along entire ventral length of valva

Archips: terminal spine-like projection present on sacculus of valva

NOTE 1: sacculus extends entire length of valva

NOTE 2: clavus of valva with teeth



E. postvittana, male genitalia and aedeagus

Cydia funebrana (Treitschke) (LEPIDOPTERA: TORTRICIDAE)

Distribution: Europe, Mediterranean

NOTE: Some European taxonomists include *Grapholita* as subgenus of *Cydia*. *Grapholita* including *funebrana* (Treitschke))has a pair of long heavy hair tufts at apex of abdomen which distinguishes this group.

Grapholita molesta: smaller in size, male has patch of pale scales along middle of termen (outer edge) of hindwing; both sexes have better defined fasciate markings than *funebrana* and a white discocellular spot on forewing at two-third length of wing near middle. Wing expanse is 11-13 mm.

Cydia funebrana: slightly larger in size, wing expanse approximately 15 mm., no white discocellular spot or patch of pale scales along middle of termen of hindwing.

C. Funebrana
male genitalia

G. molesta
male genitalia

Autographa gamma Linnaeus (LEPIDOPTERA: NOCTUIDAE)

DISTRIBUTION: Europe, Mediterranean

The subfamily Plusiinae has a number of species with a prominent silver stigma in the center of the forewing. Male genitalia of *Autographa* are quite similar. Wing expanse of *A. gamma* is 36-40 mm. Similar-looking species are *Syngrapha celsa* (Hy. Edw.) which occurs in Western U.S. and can be separated by its spined tibiae, *A. pseudogamma* (Grote) which is a boreal species occurring in Alaska and Canada, south to Maine, Michigan, South Dakota, Montana, Wyoming, Arizona, and California, (In this species the length of the aedeagus is about seven times the length of the cornutus.) and *A. californica* (Speyer) which occurs in Western U.S. to Kansas and Nebraska and resembles *A. gamma* both externally and in genitalia.

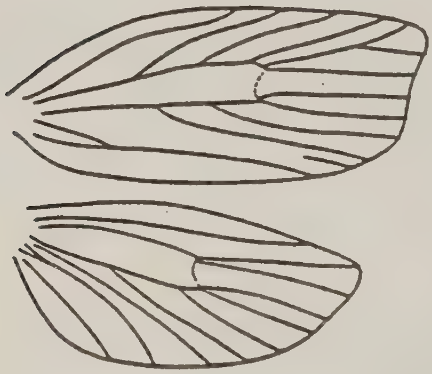
A. gamma, male genitalia

Adoxophyes orana (Fischer von Rosslerstamm) (LEPIDOPTERA: TORTRICIDAE)

Distribution: Europe

Wing expanse is 15-22 mm; sexual dimorphism pronounced in forewing

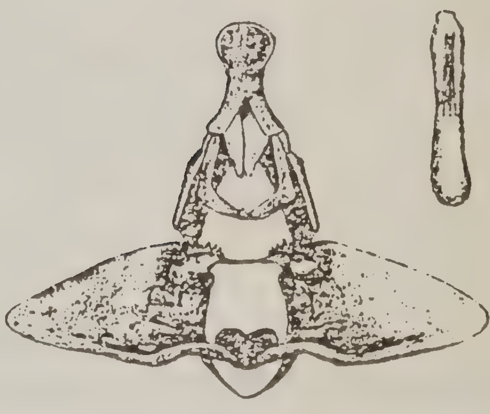
head, lateral view



wing venation

male genitalia

Resembles a large number of other moths in this family. Very closely resembles two U.S. species, Adoxophyes furcatana (Walker) and A. negundana (McDunnough) but there are slight differences in male genitalia.



Adoxophyes furcatana (Walker) male genitalia, U.S.A.

Eupoecilia ambiguella Hubner (LEPIDOPTERA: COCHYLIDAE)

DISTRIBUTION: Europe, Asia, Brazil

Forewing expanse 12-15 mm; ground color of forewings pale ochreous-white with dots and mottling of black and yellow-ochreous. Wide median fascia oblique on distal side, coloration blackish with ferruginous spots in dorsal half.

E. ambiguella, aedeagus, male genitalia

Chilo partellus (Swinhoe) (LEPIDOPTERA: PYRALIDAE)

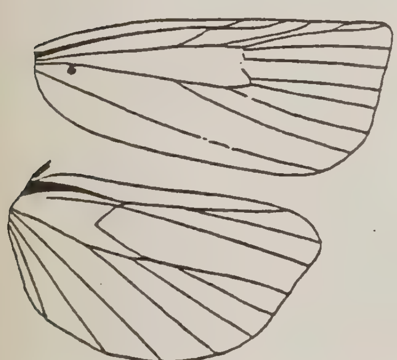
DISTRIBUTION: East Africa, India, Afghanistan

Chilo suppressalis (Walker) (LEPIDOPTERA: PYRALIDAE)

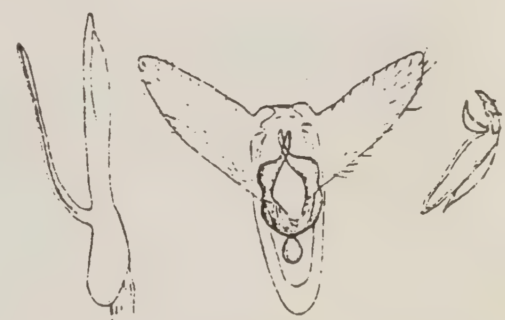
DISTRIBUTION: Spain, Asia, Hawaii

In both species ocelli above compound eye are well developed (in Diatraea no ocelli are present); forewing venation has R2 and R5 free, R3-R4 stalked; hindwing venation has M1 from upper angle of cell, M2 present. Both species can be separated by the shape of the juxta-plate (which occurs between the bases of the valvae in the center of the genitalia). Note the long arms on the juxta-plate. C. partellus has length of a forewing (not forewing expanse) varying from 7-17 mm; C. suppressalis has forewing length varying from 11-14 mm.

MALE GENITALIA: aedeagus; ventral view of juxta-plate and valvae; lateral view of uncus and gnathos



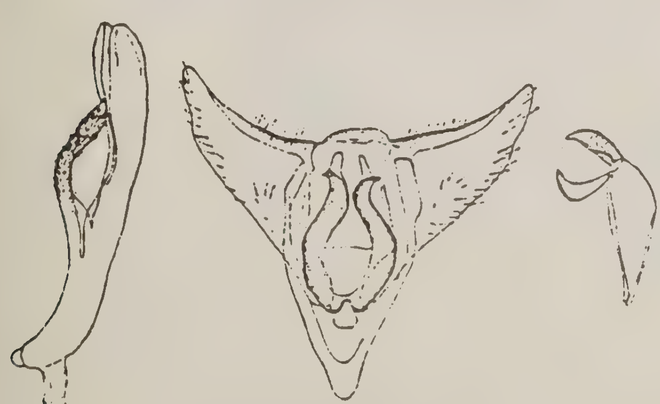
Chilo partellus (Swinhoe)



Chilo suppressalis (Walker)

Chilo demotellus Walker

Chilo erianthalis Capps



Chilo plejadellus Zincken

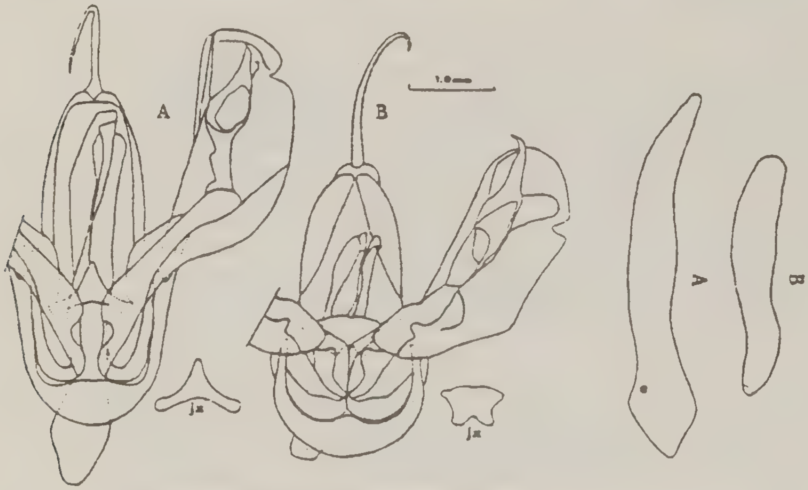
Chilo chiriquitensis (Zeller)

Spodoptera litura (Fabricius) (LEPIDOPTERA: NOCTUIDAE)

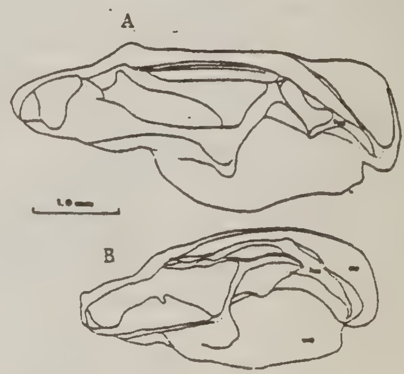
Distribution: Asia, Australia

Spodoptera littoralis (Boisduval) (LEPIDOPTERA: NOCTUIDAE)

Distribution: Africa, Mediterranean



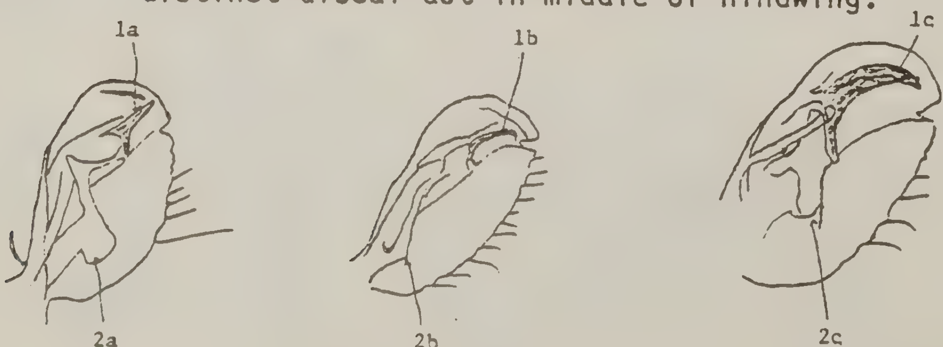
Male genitalia of *S. litura* (A) and *S. littoralis* (B). ja, juxta.



Right valvae of the male genitalia of *S. litura* (A) and *S. littoralis* (B). cu, cucullus; ha, harpe; va, valvula.

Spodoptera litura and *S. littoralis* can be confused with three U.S. species: *S. ornithogalli* (Guenee), *S. latifascia* (Walker), and *S. praefica* (Grote). Separation of the exotic species from the U.S. species can be done on the basis of the following characters of males:

litura, littoralis:	well-defined oblique band from costa of forewing through the orbicular ending at about the midpoint of the post-medial line; no discal dot in middle of hindwing; dorsal surface of thorax more variegated light brown and dark brown; hook in genitalia moderate and straight (see 1a); valva has shape as shown in 2a.
ornithogalli:	well-defined oblique band from costa of forewing through the orbicular ending at about the midpoint of the post-medial line; no discal dot in middle of hindwing; dorsal surface of thorax a more uniform brown; the hook in genitalia thinner and definitely curved (see 1b); valva has shape as shown in 2b.
latifascia:	oblique band absent and replaced by a light cream to tan-colored orbicular; no discal dot in middle of hindwing; hook in genitalia massive and curved (see 1c); valva has shape as shown in 2c.
praefica:	distinct discal dot in middle of hindwing.



S. litura right valva   *S. ornithogalli* right valva   *S. latifascia* right valva

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: P. C. Kingsley

This project was initiated in 1981 to evaluate the effects of alfalfa weevil (AW) parasitoid establishment on AW populations and subsequent changes in the local economics of growing alfalfa. The movement of principally two species, Bathyplectes anurus and Microctonus aethiopoides, from eastern areas where these species have been well established, to areas further west has been coordinated from the APHIS Biological Control Laboratory in Niles, Michigan. Recovery surveys, three years post-release, indicate widespread success of the distribution methods.

The movement of these two species has also been documented in our intensive sampling of five areas, each with six sites and five fields per site (Figure 1). Areas I-IV have been sampled for five years, whereas the Nebraska area was added to the survey in 1983.

Survey methods, as described in the 1985 Evaluation Handbook, remained the same as the previous year.

Alfalfa weevil populations in the three eastern areas (II, III, IV), as indicated by mean peak larval densities (Table 1A) and seasonal phenologies (Figures 4-6), showed upward trends in 1985 to at least twice the previous years levels. Weevil populations in these eastern areas also occurred and peaked earlier in 1985 than in previous years. In Ohio (area III) for example, larval densities peaked on Julian date 130 (May 10, Figure 7), twenty-five days earlier than in 1984 (day 155, June 4).

Table 1A. Five-year area summary for peak alfalfa weevil larval densities.

	Mean (SE) Peak Larval Density Number per 100 Sweeps				
Area	1981	1982	1983	1984	1985
O NB			262.3ab (26.5)	706.7a (164.93)	731.3b (101.25)
I MO, IA	619.5b[1] (105.0)	193.3ab (36.2)	351.7b (56.1)	253.0b (28.56)	367.7a (83.20)
II IA, IL	846.3b (199.3)	251.5ab (59.0)	207.0a (33.8)	345.4b (77.43)	694.2b (72.01)
III OH	92.1a (22.3)	97.5a (22.9)	161.8a (8.4)	245.8b (31.12)	686.2b (112.16)
IV NJ, PA	218.1a (39.9)	381.7b (87.9)	171.1a (34.8)	52.8b (13.67)	268.7a (46.34)

Table 1B. Five-year area summary for peak alfalfa weevil parasite species diversity.

	Mean (SE) Number of Parasite Species/Site				
Area	1981	1982	1983	1984	1985
O NB			1.5a (0.22)	1.5a (0.22)	2.3a (0.33)
I MO, IA	1.7a (0.21)	2.3a (0.21)	2.3a (0.33)	2.0a (0.00)	3.2ab (0.31)
II IA, IL	2.2a (0.17)	2.5a (0.33)	2.5a (0.34)	3.0b (0.45)	3.8b (0.31)
III OH	3.5b (0.22)	3.8b (0.31)	3.5b (0.34)	3.3b (0.24)	4.2b (0.31)
IV NJ, PA	4.5c (0.29)	4.0b (0.26)	3.8b (0.17)	3.0b (0.00)	4.2b (0.17)

1/ Values in columns followed by the same letter are not significantly different at the 95 percent level of confidence [Newman-Keuls multiple comparison test].

Table 2A. Five year summary of overall percent parasitism of alfalfa weevil adults.

Area	1981	1982	1983	1984	1985
			% Adult Parasitism (Total Parasitized/Total Dissected)		
O NB			0.04a1/ (1/2630)	0.07a (2/3074)	0.05a (20/4000)
I MO, IA	02.3a (126/5378)	13.2a (313/2374)	16.5b (724/4392)	22.7d (467/2054)	34.3d (1411/4118)
II IA, IL	39.1b (1849/4724)	35.9c (598/1666)	35.1c (478/1361)	17.2bc (293/1707)	27.4c (1635/5960)
III OH	32.0b (562/1759)	26.6b (287/1081)	18.3b (421/2295)	20.2dc (424/2095)	26.3c (1425/5422)
IV NJ, PA	26.2b (107/408)	32.6bc (587/1799)	19.1b (230/1026)	14.0b (103/734)	20.7b (694/3361)

Table 2B. Five year summary of overall percent parasitism of alfalfa weevil larvae.

Area	1981	1982	1983	1984	1985
			% Larval Parasitism (Total Parasitized/Total Dissected)		
O NB			24.8c1/ (1209/4871)	22.2b (1102/4957)	22.3c (1495/6720)
I MO, IA	14.4c (976/6760)	18.1a (358/1983)	13.8a (932/6773)	15.2a (720/4725)	20.1b (1329/6600)
II IA, IL	08.1a (586/7266)	40.0c (1096/2739)	29.0d (1672/5771)	25.5cc (1295/5078)	17.2a (1051/6124)
III OH	11.6b (452/3900)	26.0b (903/3609)	15.7b (1065/6778)	22.6b (1204/5337)	21.0b (1769/8410)
IV NJ, PA	30.9d (889/2881)	26.3b (1290/4904)	27.1cd (1443/5331)	14.1a (461/3271)	23.3c (1645/7058)

1/ Values in columns followed by the same letter are not significantly different at the 95 percent level of confidence [Chi-square].

The three western areas continued to show steady increases in parasitoid establishment (Table 1B, Figures 8-10). In Illinois and Ohio (areas II and III), this increase is due largely to the increased recoveries of Bathyplectes anurus (Figure 8). From 1981 to 1985, the percentage of fields from which this species was recovered, increased consistently from 37.9% to 70% in Ohio, and from 0% to 40% in Illinois. In Ohio, this has apparently been at the expense of its congener, B. curculionis (Figure 11). Microctonus aethiopoides has shown a similar increase in recoveries in Missouri and Iowa from 36% of the fields in 1981, to 100% in 1984 and 1985 (Figure 10). In addition to the increases in field recoveries, seventeen new county records were recorded from evaluation samples this year, compared to four in 1984.

In terms of parasitism rates, B. anurus has yet to have an impact in Illinois, as only 0.8% of the larvae were parasitized by this species (Figure 12). Microctonus aethiopoides, however, has continued to increase its importance in area I, from parasitizing 6% of the adult weevils in 1981 to 34.3% in 1985.

Data on diseased adults and larvae were collected, as in 1984, at the time of dissections. Recovery and disease rates for the two years are presented in Table 3.

Table 3. Field recovery and infection rates of diseased alfalfa weevil adults and larvae during the 1984 and 1985 AW Evaluation Surveys.1/

Area	% of fields2/ with diseased weevils		% of larvae diseased		% of adults diseased	
	1984	1985	1984	1985	1984	1985
NE	90.0	96.7	9.0	10.1	4.4	1.4
MO, IA	93.3	100.0	10.2	10.8	1.9	0.9
IL	86.7	76.6	7.0	6.7	1.3	1.4
OH	63.3	83.3	3.0	2.4	0.4	0.8
PA, NJ	64.0	66.7	8.9	1.9	3.4	0.7

1/ Determined as weevils were being dissected for parasitism data.
2/ 30 fields per area, except 1984 PA, NJ with 25.

Additional and more detailed information on AW densities and parasitism rates by species, summarized on a site and area basis, can be found in five annual archival data reports.

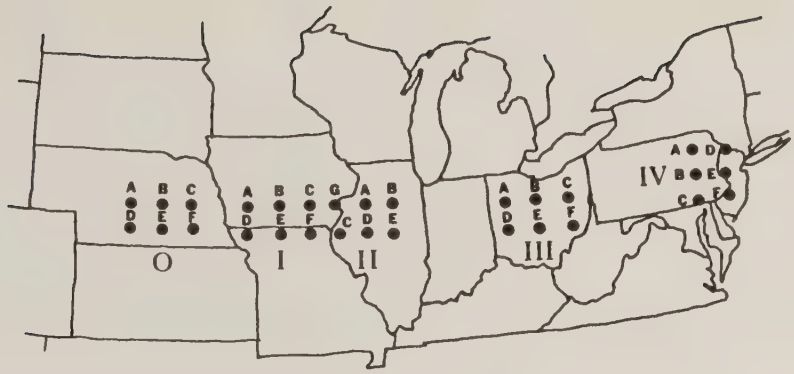


Figure 1. 1986 AW Evaluation Areas and Sites
(5 Areas, 6 Sites/Area, 5 Fields/Site).

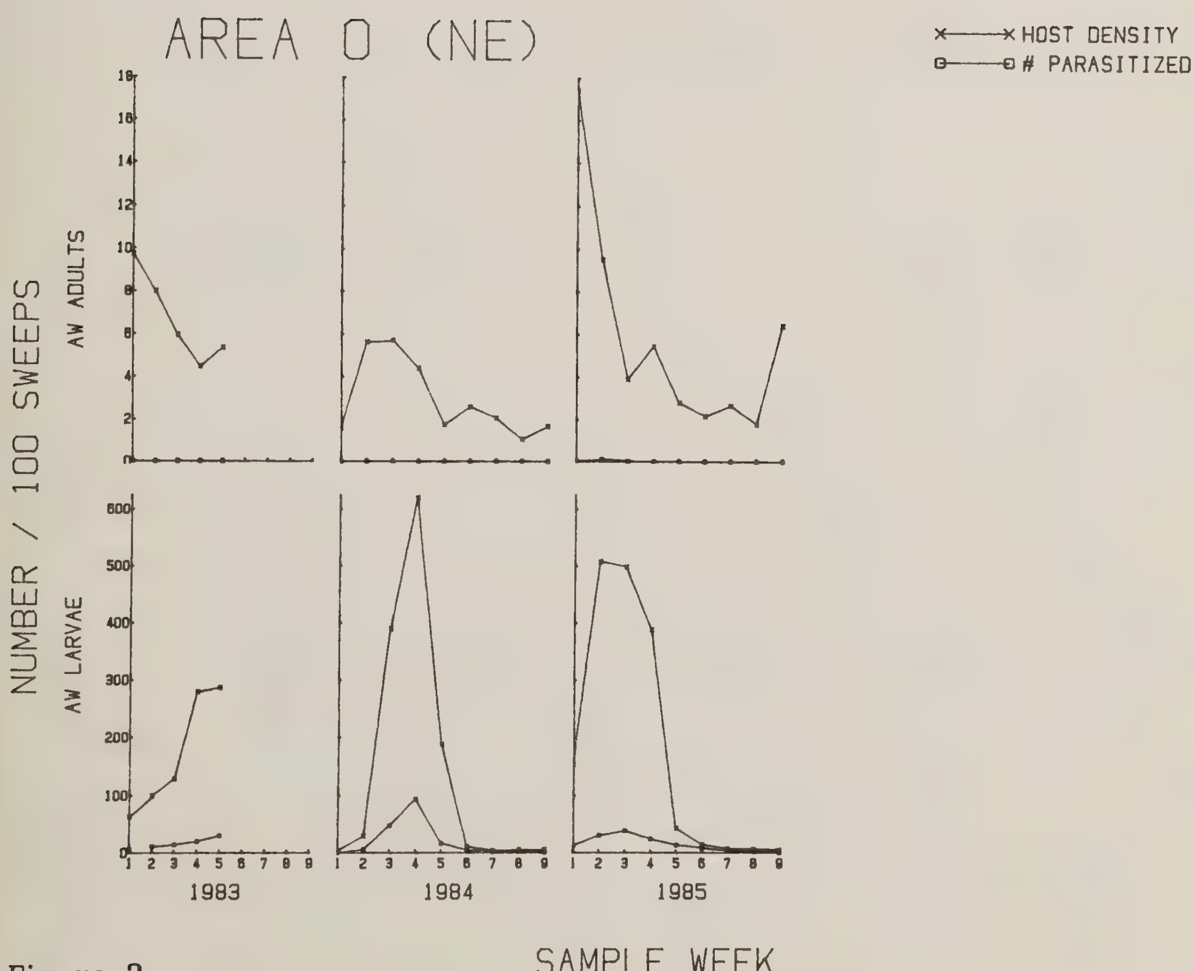


Figure 2.

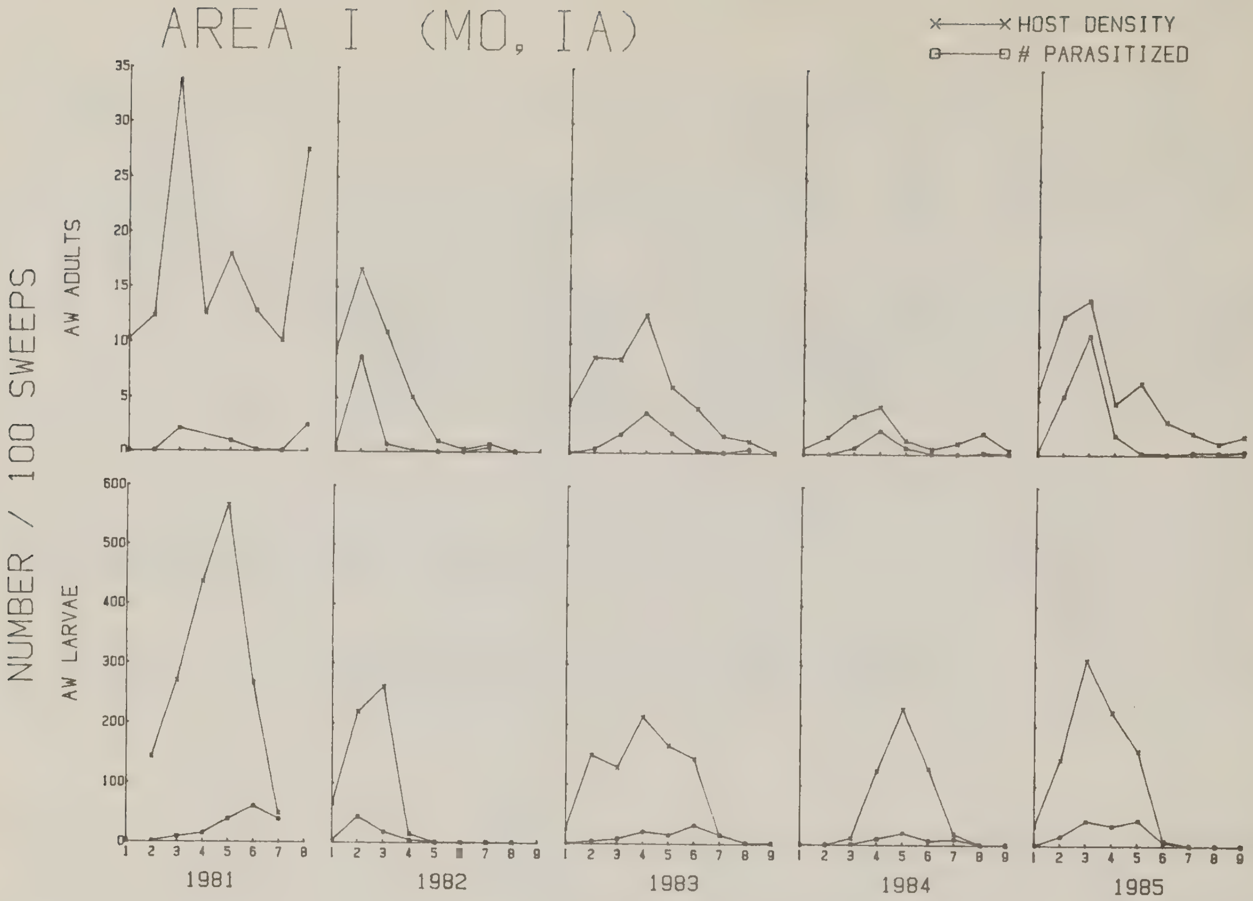


Figure 3. SAMPLE WEEK

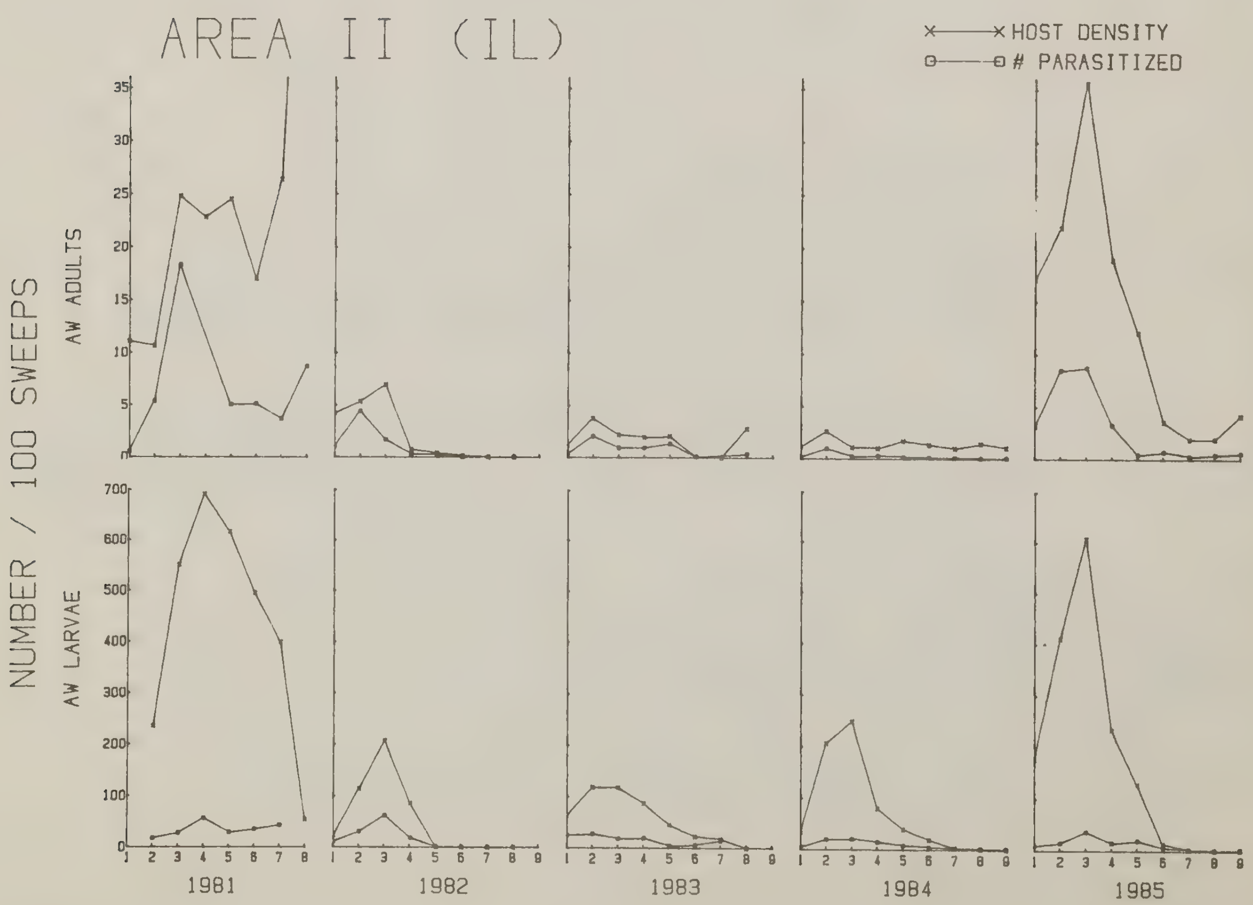


Figure 4. SAMPLE WEEK

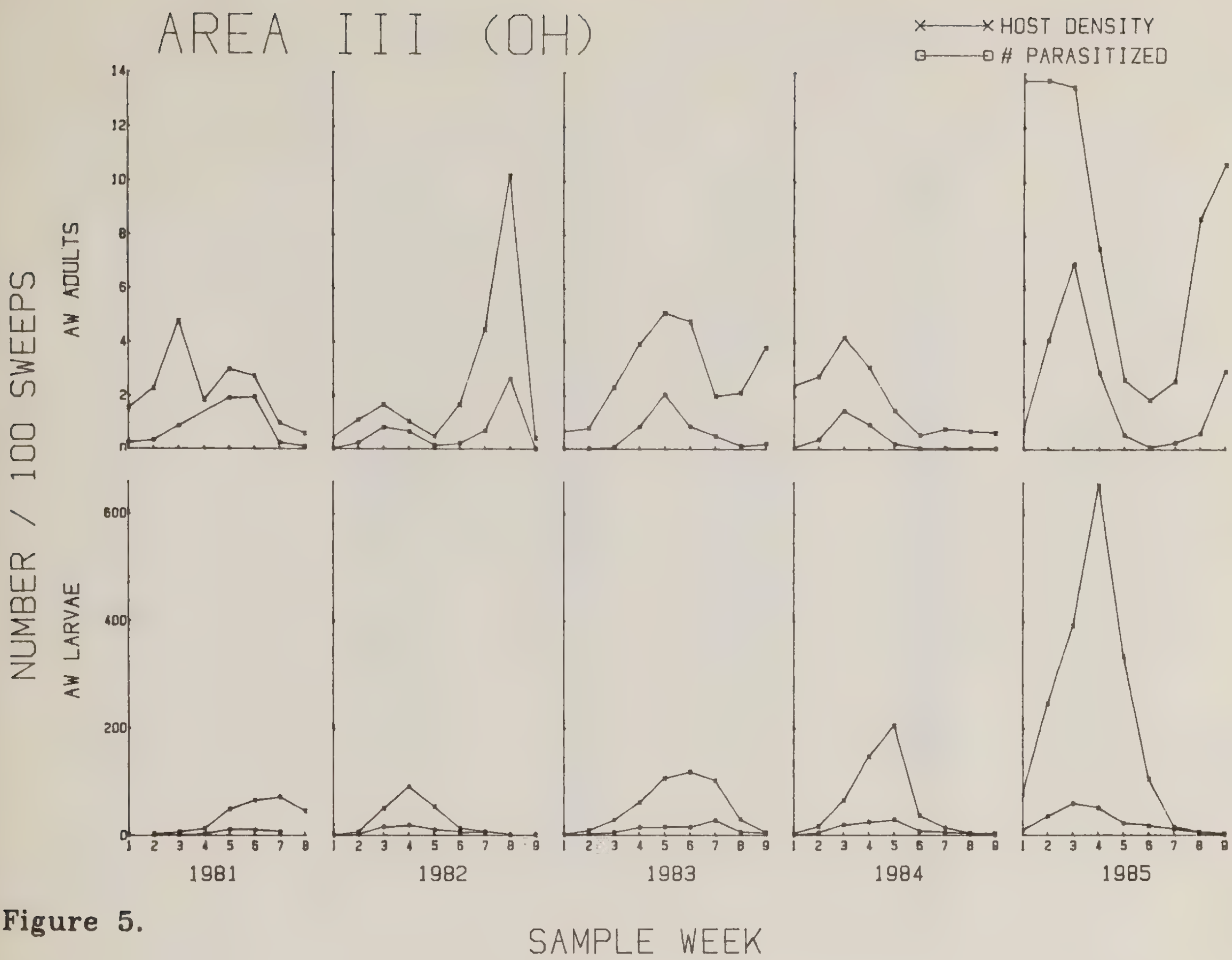


Figure 5.

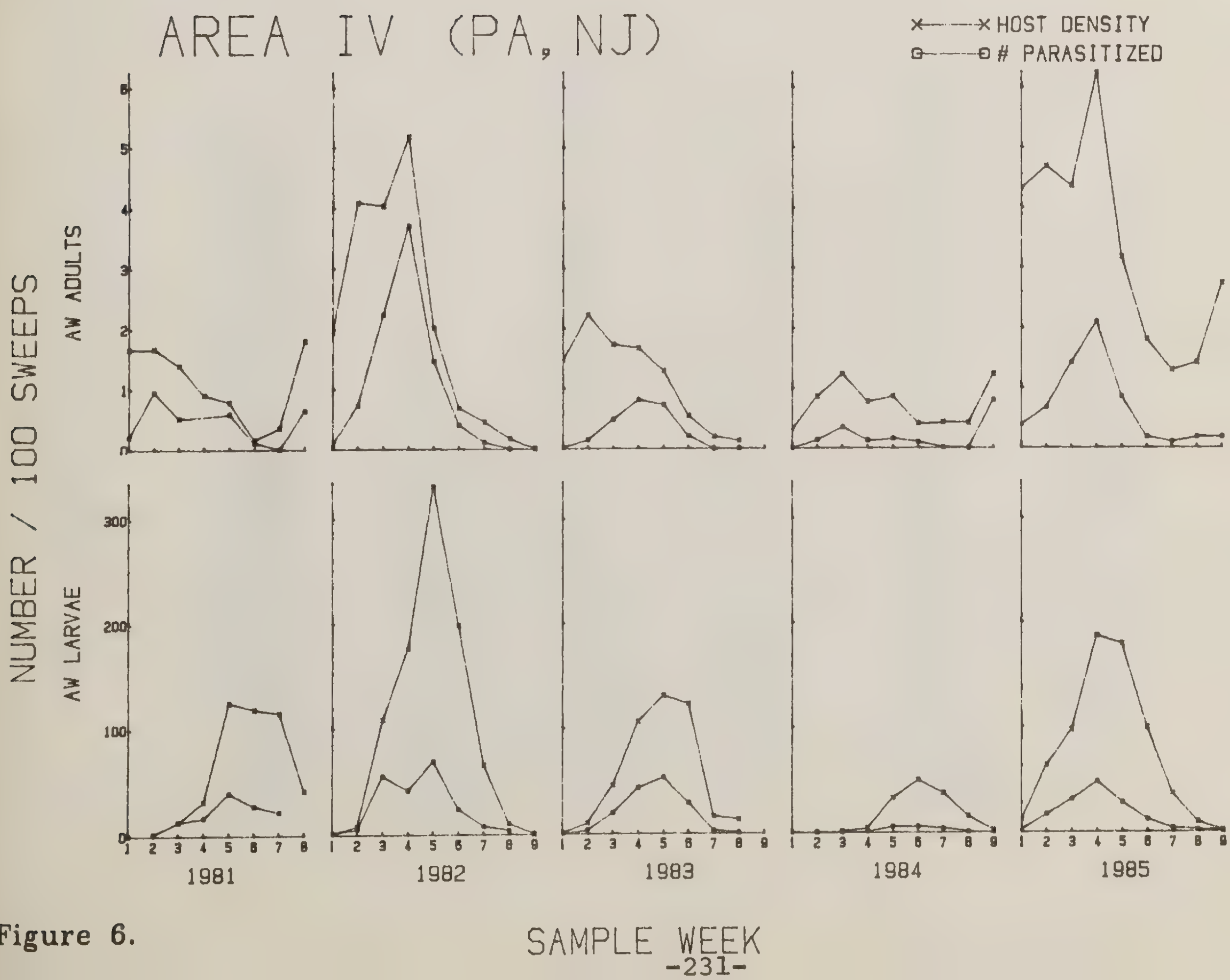


Figure 6.

Average Accumulated Degree-Days from six Ohio AW Evaluation Sites.

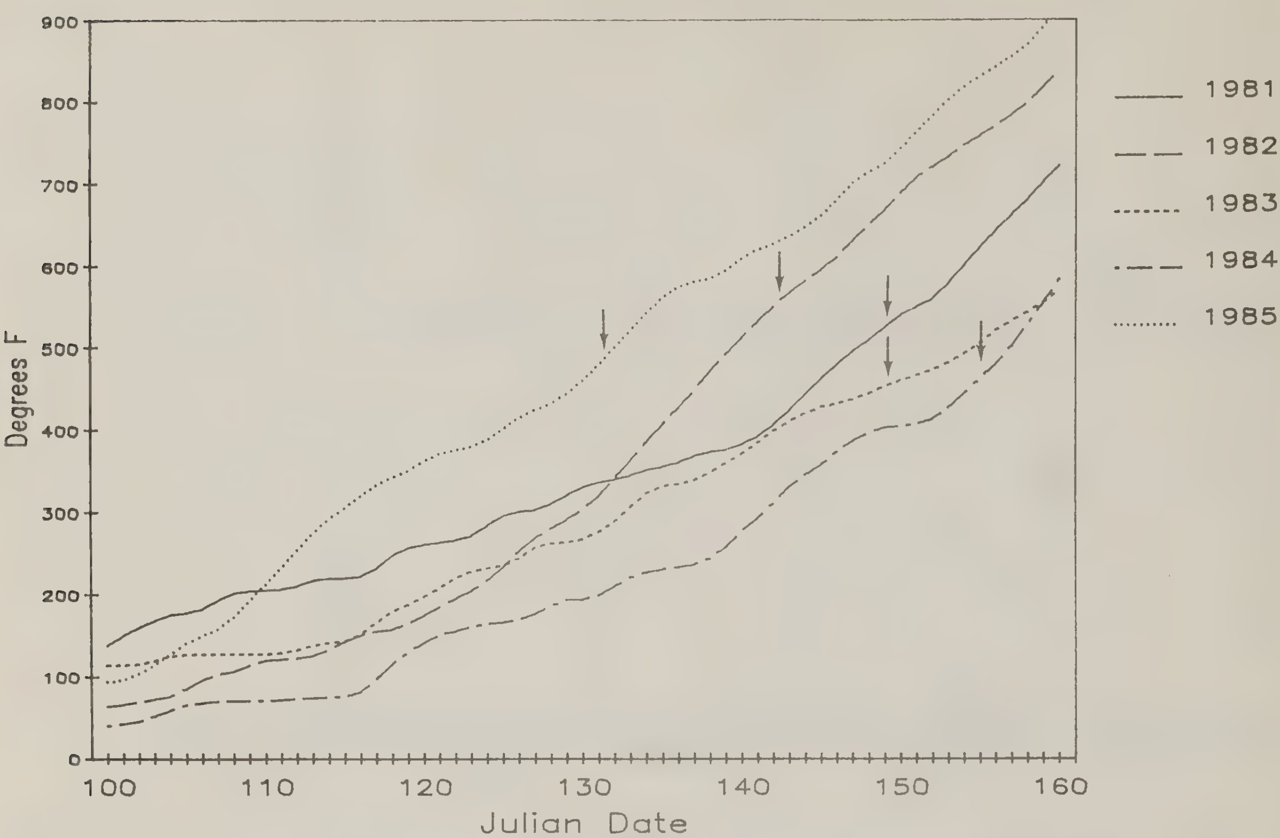


Figure 7.

AW Parasitoid Recovery Rates
Bathyplectes anurus

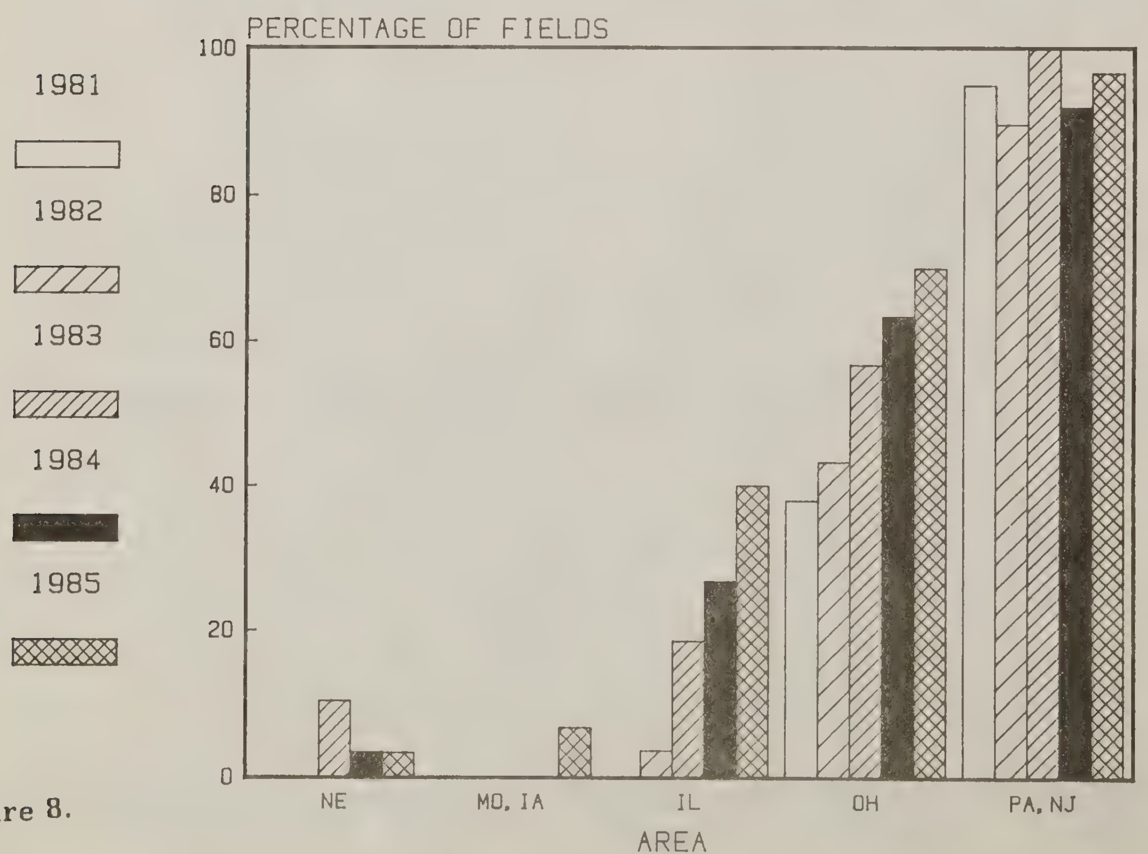


Figure 8.

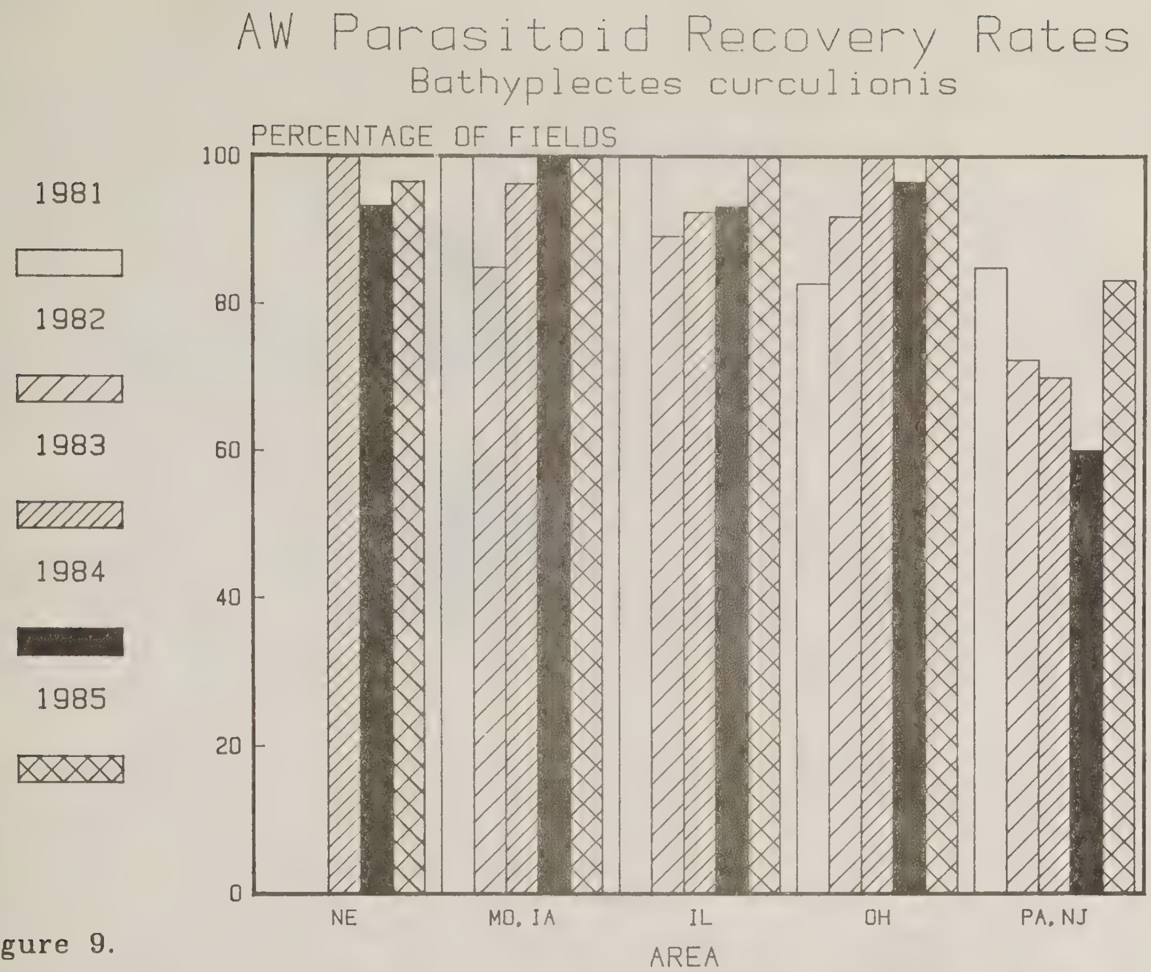


Figure 9.

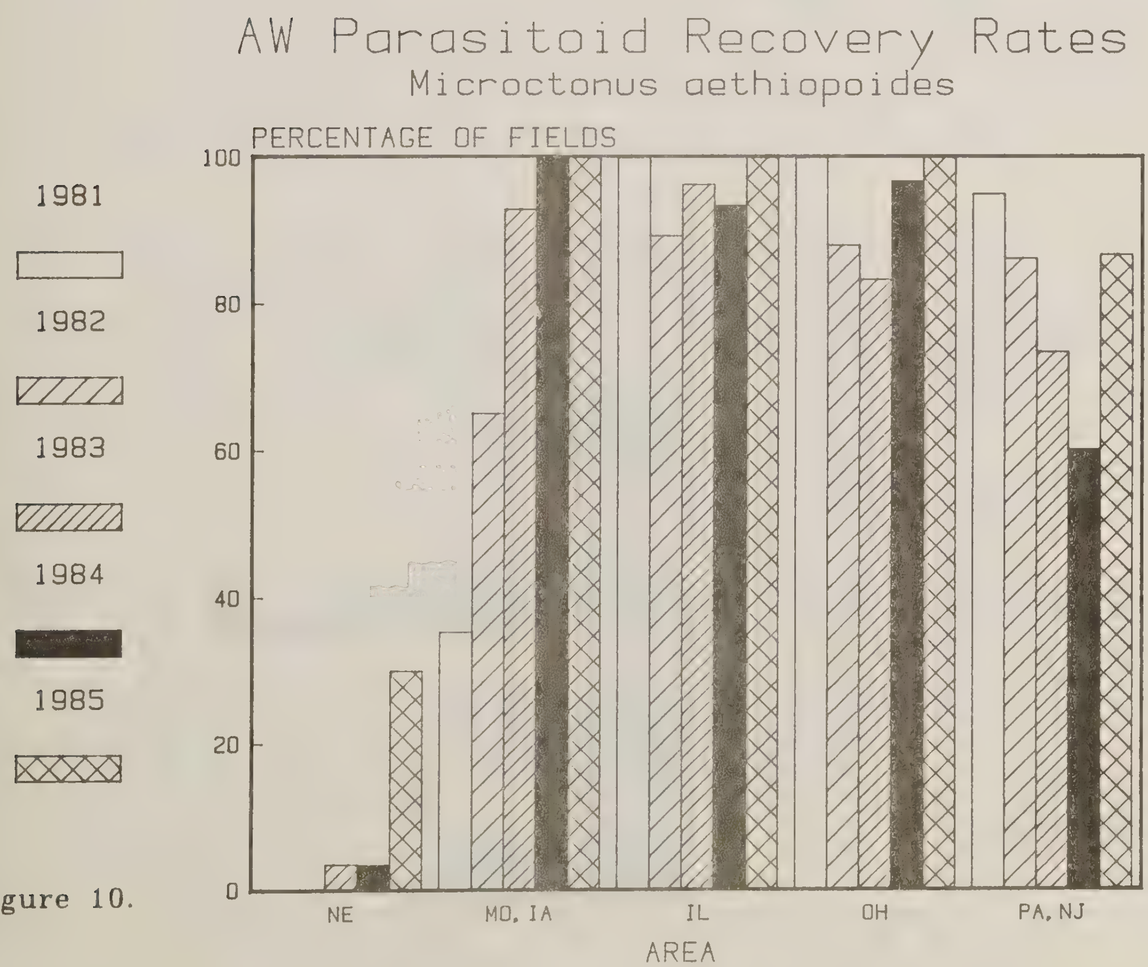


Figure 10.

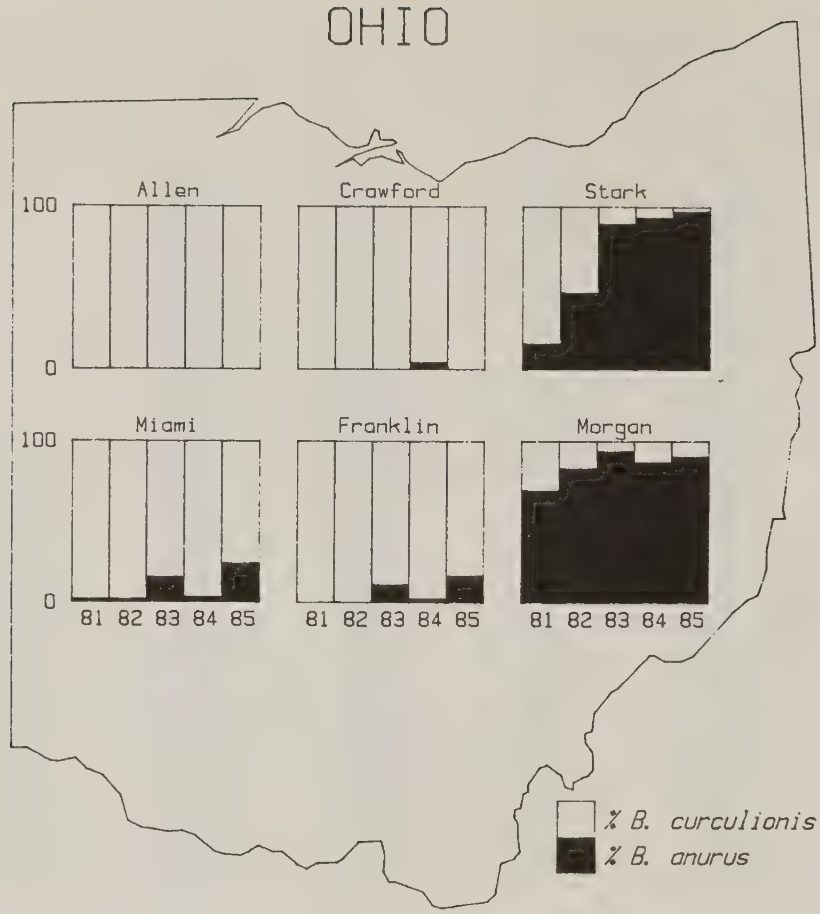


Figure 11. Relative frequencies of the two AW parasitoids Bathyplectes anurus and B. curculionis reared from six evaluation sites in Ohio, by year. The total number reared from each year was 1495, 2432, 2426, 2478, and 5071 respectively.

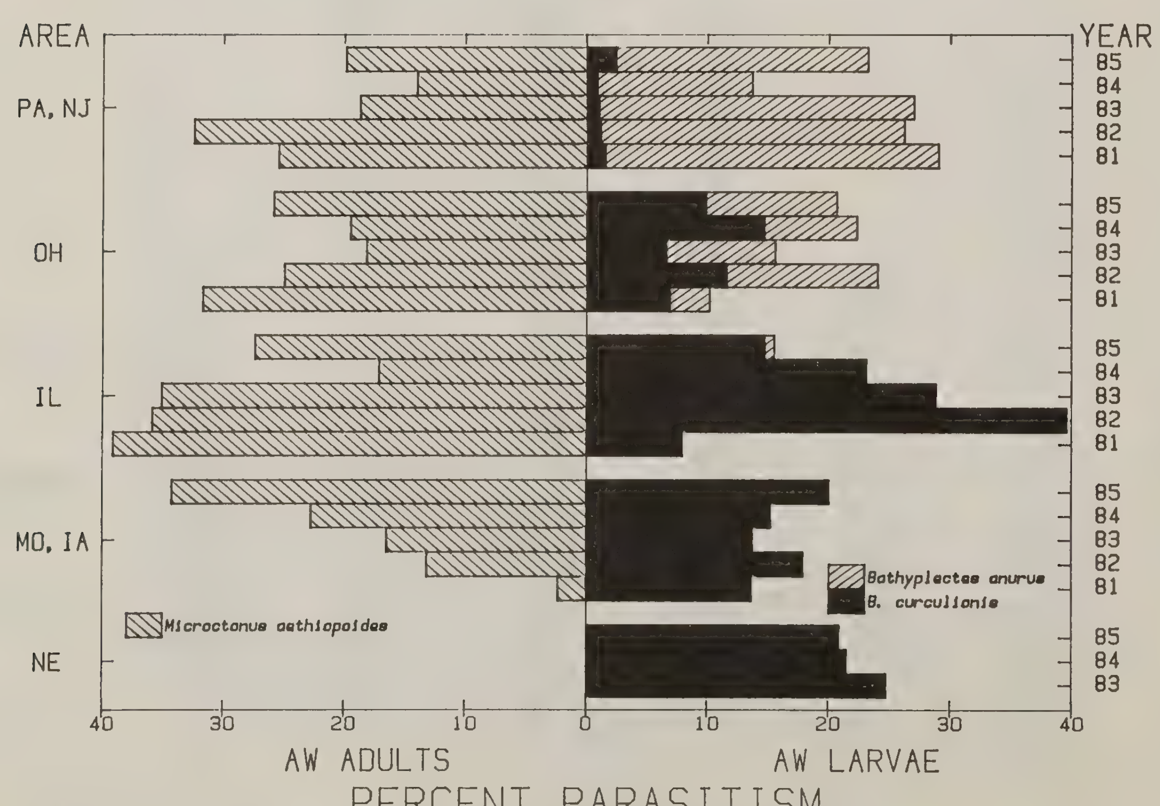


Figure 12.

Project Number: AW 2.1.1
Project Title: Alfalfa Weevil Rearing
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leader: Philip C. Kingsley

This project was initiated in 1982 in response to a need in the AW Parasite Redistribution Program for an artificial diet. Such diet could hold field collected parasitized AW larvae in transport to release fields and be useful in the parasite recovery survey that follows releases.

During the first two years of this project we developed a high wheat germ diet, with acetone extracted alfalfa powder added as a feeding stimulant, that successfully supported parasite production from AW larvae and adults. The following experiments were conducted in 1984 and 1985 to further refine rearing techniques and test their feasibility for incorporation into the Redistribution Program. All tests were done in cooperation with the Niles Biocontrol Laboratory.

A second experiment (see AW 2.1.1, 1983 Interim Report) to test the effects of an artificial diet for use in the transportation of large numbers of field collected parasitized AW larvae on parasite survival was conducted in 1984. On May 24, 20 thousand larvae were collected in a Michigan field by Niles personnel. The larvae were divided between two five gallon cherry tins, one with artificial diet (mixed here at the Otis Methods Development Center on May 22) incorporated on a substrate of excelsior and the other with alfalfa. After being flown here on May 25, three subsamples of 300 larvae were taken from each treatment and placed on field grown afalfa in paper grocery bags. Tins were held at room temperature for two days at which time two additional subsamples of 200 larvae were removed and placed on alfalfa as before. Larvae were fed until all weevils and parasites had spun cocoons. Adult weevils and Bathyplectes cocoons were counted during the second week in July. Results are as follows:

Treatment	Days After Collection	N	Adult Survival	Bathyplectes Production	Overall Survival
Alfalfa	2	900	32.6% a 1/	22.7% a	55.4% a
Diet	2	900	32.3% a	20.1% a	52.4% a
Alfalfa	4	600	14.6% c	2.2% c	16.8% c
Diet	4	600	24.8% b	8.0% b	32.8% b

1/ Those frequencies followed by the same letter are not significantly different at the .05% level (Chi-square test).

Among those larvae held for two days there were no significant differences among the parameters tested, indicating no adverse affects from diet. When larvae were held on diet and alfalfa for an additional two days, however, a significant amount of mortality occurred and to a greater extent to those larvae held on alfalfa.

The resulting Bathyplectes anurus (Ba) cocoons from the low density treatments were sent to Niles to look for adverse affects on F_1 survival and fecundity. After breaking diapause, emerging females were exposed to 50 AW larvae for 24 hours. These larvae were then dissected 2-3 days later to record parasitism and the number of progeny produced per female. In addition, each female Ba was then dissected to count the number of oocytes remaining the the ovaries.

Treatment	N	Percent Emergence	Percent Females	Percent Parasitism	Mean (SD) Number of Eggs Laid / Female	Mean (SD) Number of Oocytes / Female
Alfalfa	131	79.4	57.6	53.3	49.0 (36.8)	169.9 (105.37)
Diet	103	54.0	43.4	67.6	69.6 (48.39)	114.2 (42.24)
		**	NS	NS	NS	NS

The only significant difference between the parameters tested was in the percentage of wasps emerging from cocoons. Those wasps produced from diet-fed larvae had a 25% higher mortality rate than those from alfalfa-fed larvae.

Since the large scale movement of parasitized hosts was winding down in 1984, we switched emphasis from utilizing diet in the transport of parasites to incorporating the technology into the parasite recovery aspect of the program. Three years post-release, fields are surveyed for the released parasite species. The surveyor collects up to 300 larvae, places them into a paper grocery bag with some alfalfa and sends them to a processing laboratory where additional alfalfa is added to the bags until parasites emerge. In the case of adult weevils, special plastic adult parasite rearing containers are used. In this next experiment, we attempted to incorporate diet into the recovery survey.

Three treatments were replicated three times; alfalfa fed, alfalfa fed and transported then switched to diet, and diet fed. Adults were shipped at a density of 100 per carton while larvae were tested at two densities, 500 and 250 per container. Diet was mixed here and shipped to Niles. For the larvae, diet was incorporated onto an excelsior substrate in one quart ice cream containers. Diet for adult weevils was coated onto a coarse plastic screen and placed in the adult rearing containers. Larvae and adults were field collected in Michigan and placed on either alfalfa (in grocery bags) or diet. Upon receipt here, two days later, half of the alfalfa fed weevils were transferred to diet. Results were as follows:

LARVAE

Treatment	Density	N	Adult Survival	Parasite Production	Overall Survival
Alfalfa	250	750	(364) 48.5% bc	(67) 8.9% a	(431) 57.5% ab
	500	1500	(816) 54.4% a	(92) 6.1% b	(908) 60.5% a
Alfalfa to Diet	250	750	(385) 51.3% ab	(35) 4.7% c	(420) (56.0% abc
	500	1500	(726) 48.4% bc	(70) 4.7% c	(796) 53.1% bc
Diet	250	750	(348) 46.4% bc	(26) 3.5% c	(374) 49.9% cd
	500	1000	(424) 42.4% c	(42) 4.2% c	(466) 46.6% d

ADULTS

Treatment	Density	N	Parasite Production
Alfalfa	100	300	(136) 45.3% b
Alfalfa to Diet	100	300	(173) 57.7% a
Diet	100	300	(141) 47.0% ab

Higher larval densities did not significantly reduce overall survival in the diet treatments, although at a density of 500 larvae per paper bag, parasite production was adversely affected in the alfalfa fed larvae. Most importantly however, there was a significant decrease in the number of parasites produced by those larvae fed either diet after alfalfa or diet alone. This reduction was most likely due to the diet drying out and could be remedied by adding additional fresh diet or possibly by a modification of the container. Under these conditions, Microctonus aethiopoides production from adult weevils switched to diet was significantly higher than those adults fed alfalfa alone. In addition, adults fed diet alone produced similar numbers of the parasite as alfalfa fed adults.

The results from this experiment indicate that with slight modifications, diet could be successfully utilized as part of the recovery survey in the rearing of parasites from field collected larvae and adults.

Project Number: AW 3.1.1
Project Title: Alfalfa Weevil Strain Identification Project
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: P. C. Kingsley

Alfalfa weevil adults, reared from larvae, collected from 24 western insectaries were sent to the Mission Biological Control Laboratory for electrophoresis. A method for identifying strains has been developed by Jeanne Romero-Andreas through a cooperative agreement with the Otis Methods Development Center and will be used at the Mission Laboratory. Results from this work will be reported in the next Progress Report.

Project Number: MBB 1.1.4
Project Title: Direct Release of *Pediobius foveolatus* (Pf) into Soybean Fields for Control of Mexican Bean Beetle (MBB)
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leaders: O. T. Forrester

Work on this project has been terminated because adequate MBB populations can not be located.

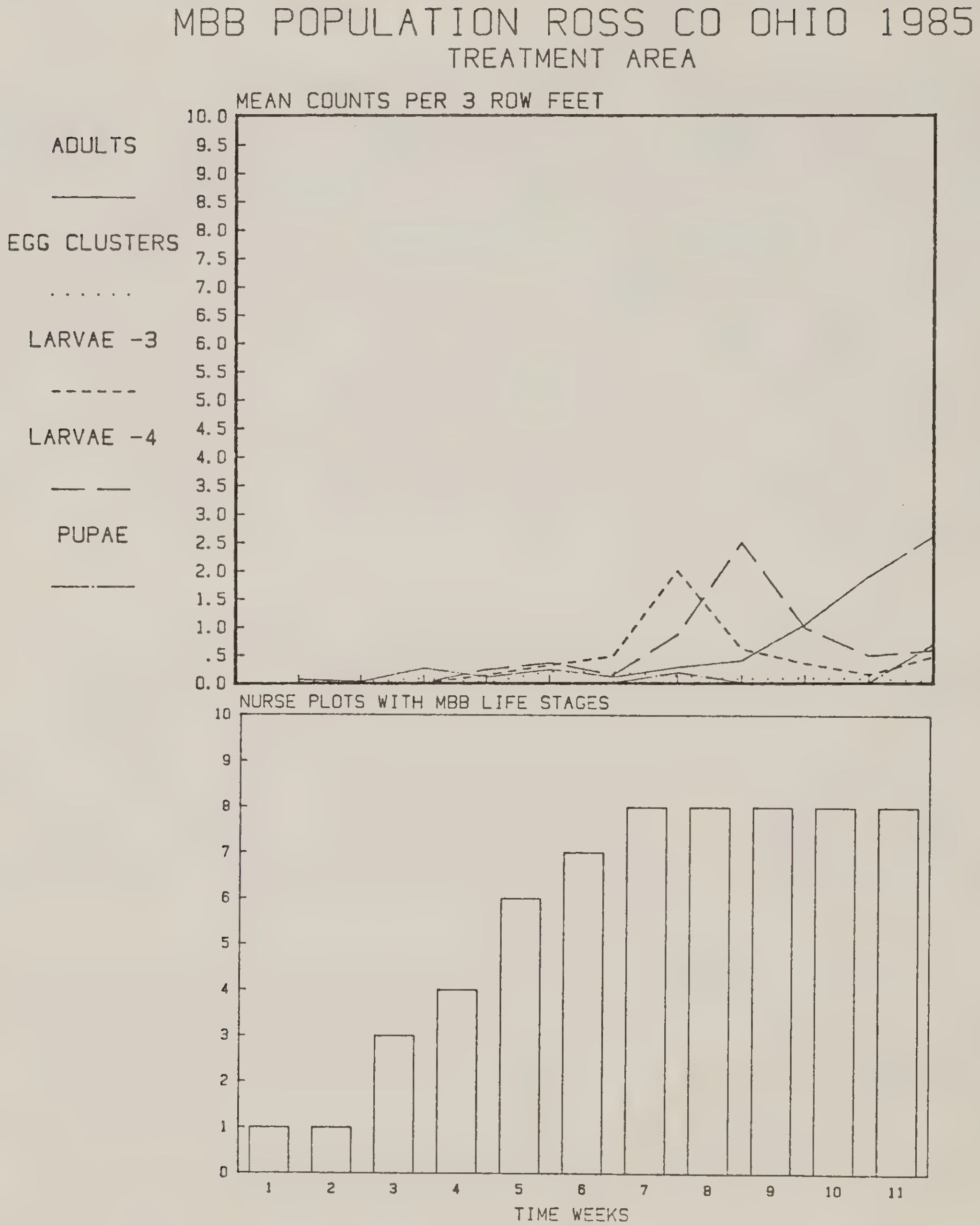
Project Number: MBB 4.1.1
Project Title: Biological Control of Mexican Bean Beetle (MBB) Over Large Areas with _Pediobius foveolatus_ (Pf)
Report Period: October 1, 1984 - September 30, 1985
Report Type: Final
Project Leader O. T. Forrester

This reports the results of a two year project designed to test the hypothesis that high parasitization rates of larval MBB result in a lower over-wintering population and a reduced infestation level the following season. (Detailed project description in last Progress Report.) The MBB population in Ohio has been on a downward trend since 1981 and has caused difficulty in locating an adequate work area. A modification of the original plan was devised to ease the effect of the collapsing population. A small nurse plot 15' x 25' was planted in a corner of each of the first 10 soybean fields in the proximity of the center nurse plot of last season's study sites. These nurse plots were in the center 4 square miles of last years 36 square mile release areas. This should have minimized the impact of immigration on this years results. Intensive weekly sampling was conducted in the nurse plots and the adjoining soybean field. The results of these samples are shown in Figures 1-7. MBB populations were extremely low this season. The only MBB populations detected were in the nurse plots.

Figures 1-3 depict the seasonal development of MBB population in the Scioto River Valley nurse plots. These were the highest populations observed. T-tests were run comparing total and maximum over-wintered adults and over-wintered plus first generation adults. No significant difference was indicated. Figures 4-7 depict the seasonal development of MBB populations in Clark and Preble Counties. T-tests were run on data groups similar to the Scioto area. No significant difference was indicated. In all cases, the incidence of MBB occurrance in nurse plots was similar in sample areas, regardless of whether or not parasites had been released the previous year, suggesting the over-wintering populations were not different.

The lack of statistical significance in this test may be attributed to working in a collapsing population. MBB has not been of economic importance during the two years of this study. Based on these results, one cannot conclude that _Pediobius foveolatus_ is of value in controlling MBB in soybeans. It is difficult, if not impossible, to make this conclusion when working with an insect that is not causing economic damage at the time of the study. The concept tested is a valid concept and should be tested again.

Figure 1.


MBB POPULATION ROSS CO OHIO 1985
TREATMENT AREA
MEAN COUNTS PER 3 ROW FEET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
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5.0
4.5
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3.5
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2.5
2.0
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NURSE PLOTS WITH MBB LIFE STAGES
10
9
8
7
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4
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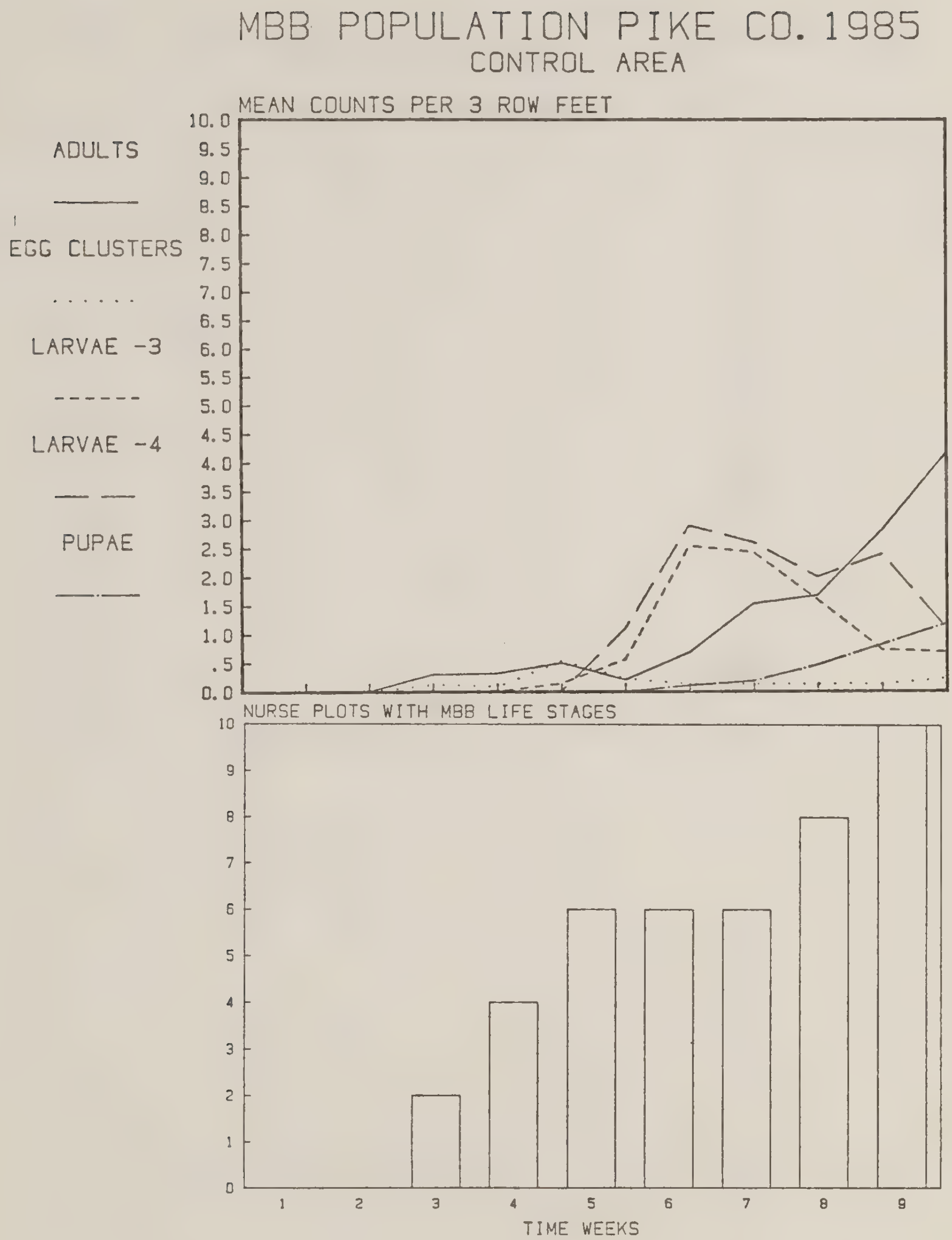
1
0
1 2 3 4 5 6 7 8 9 10 11
TIME WEEKS

Figure 2.


MBB POPULATION PIKE CO. 1985
CONTROL AREA
MEAN COUNTS PER 3 ROW FEET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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NURSE PLOTS WITH MBB LIFE STAGES
10
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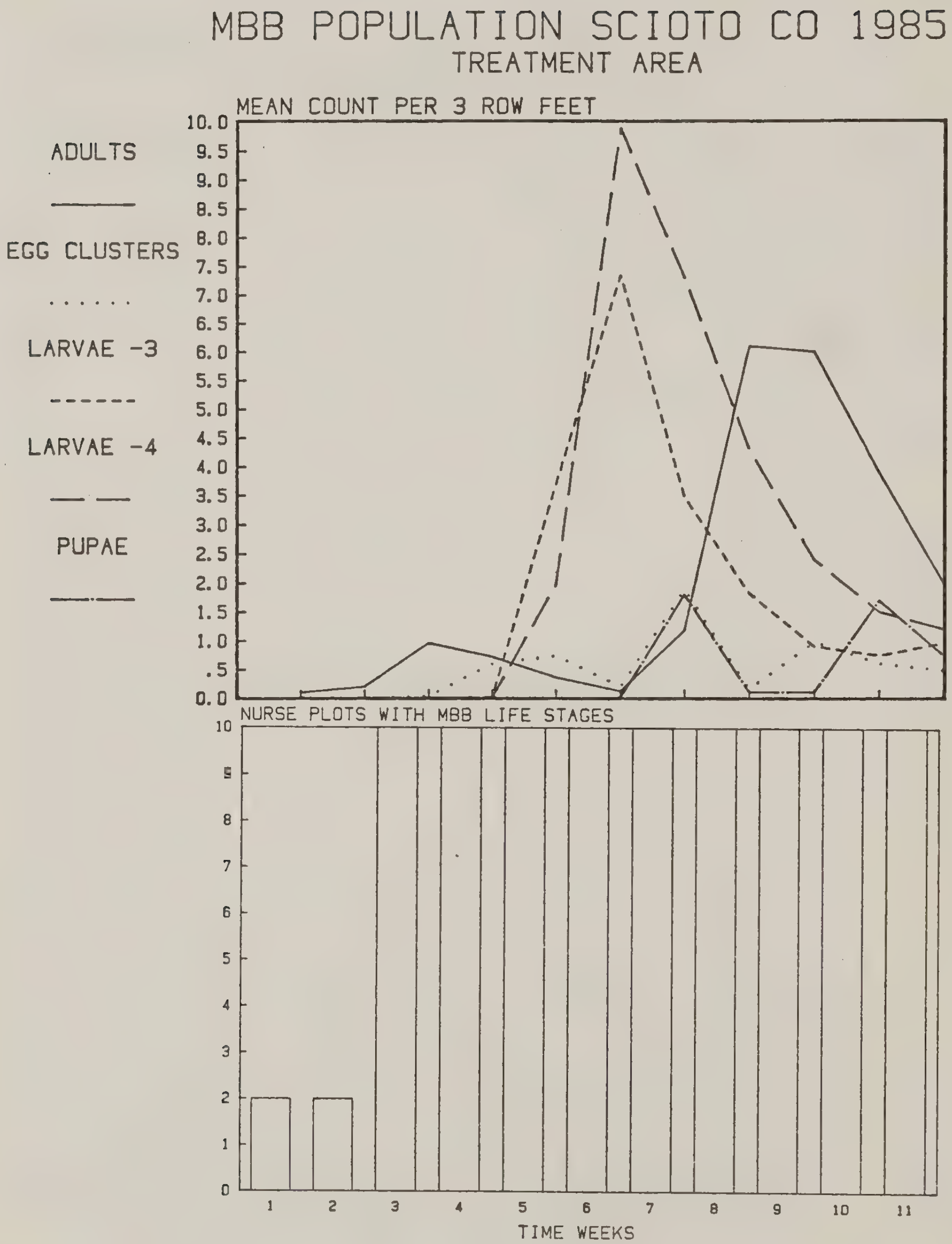
TIME WEEKS

Figure 3.


MBB POPULATION SCIOTO CO 1985
TREATMENT AREA
MEAN COUNT PER 3 ROW FEET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
7.5
7.0
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2.5
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NURSE PLOTS WITH MBB LIFE STAGES
10
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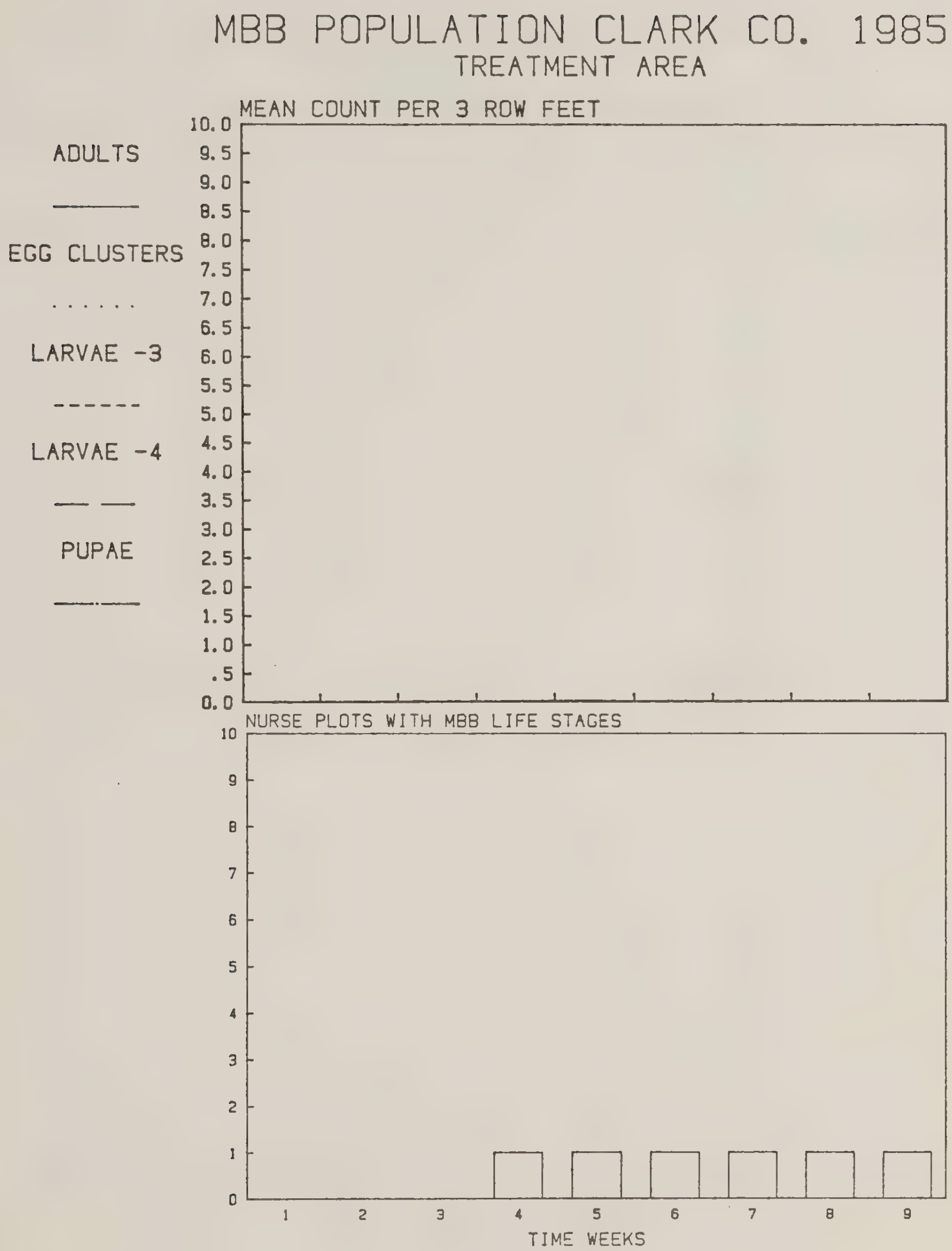
TIME WEEKS

Figure 4.



MBB POPULATION CLARK CO. 1985
TREATMENT AREA
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
MEAN COUNT PER 3 ROW FEET
10.0
9.5
9.0
8.5
8.0
7.5
7.0
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5.0
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4.0
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NURSE PLOTS WITH MBB LIFE STAGES
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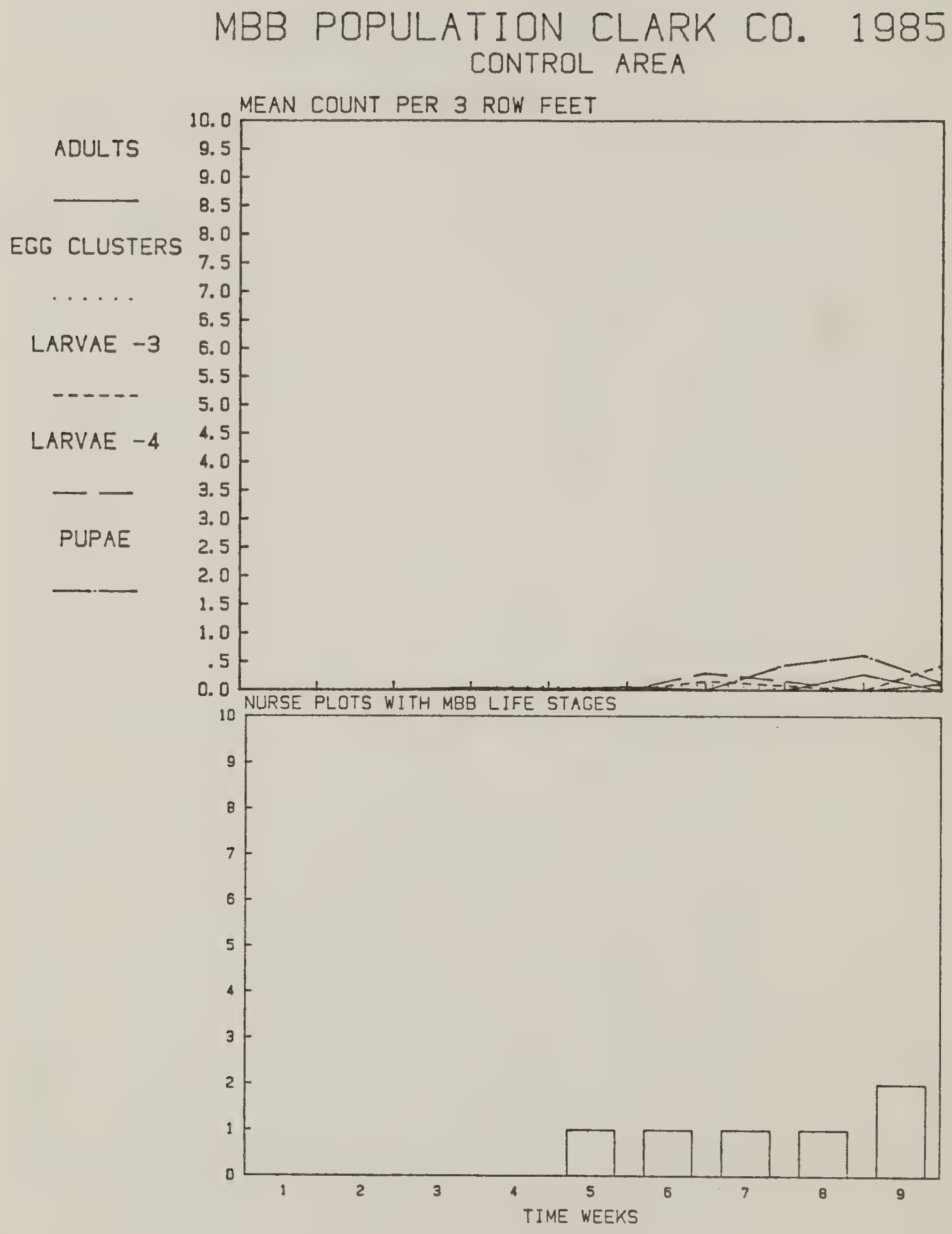
TIME WEEKS

Figure 5.



MBB POPULATION CLARK CO. 1985
CONTROL AREA
MEAN COUNT PER 3 ROW FEET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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4.5
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NURSE PLOTS WITH MBB LIFE STAGES
10
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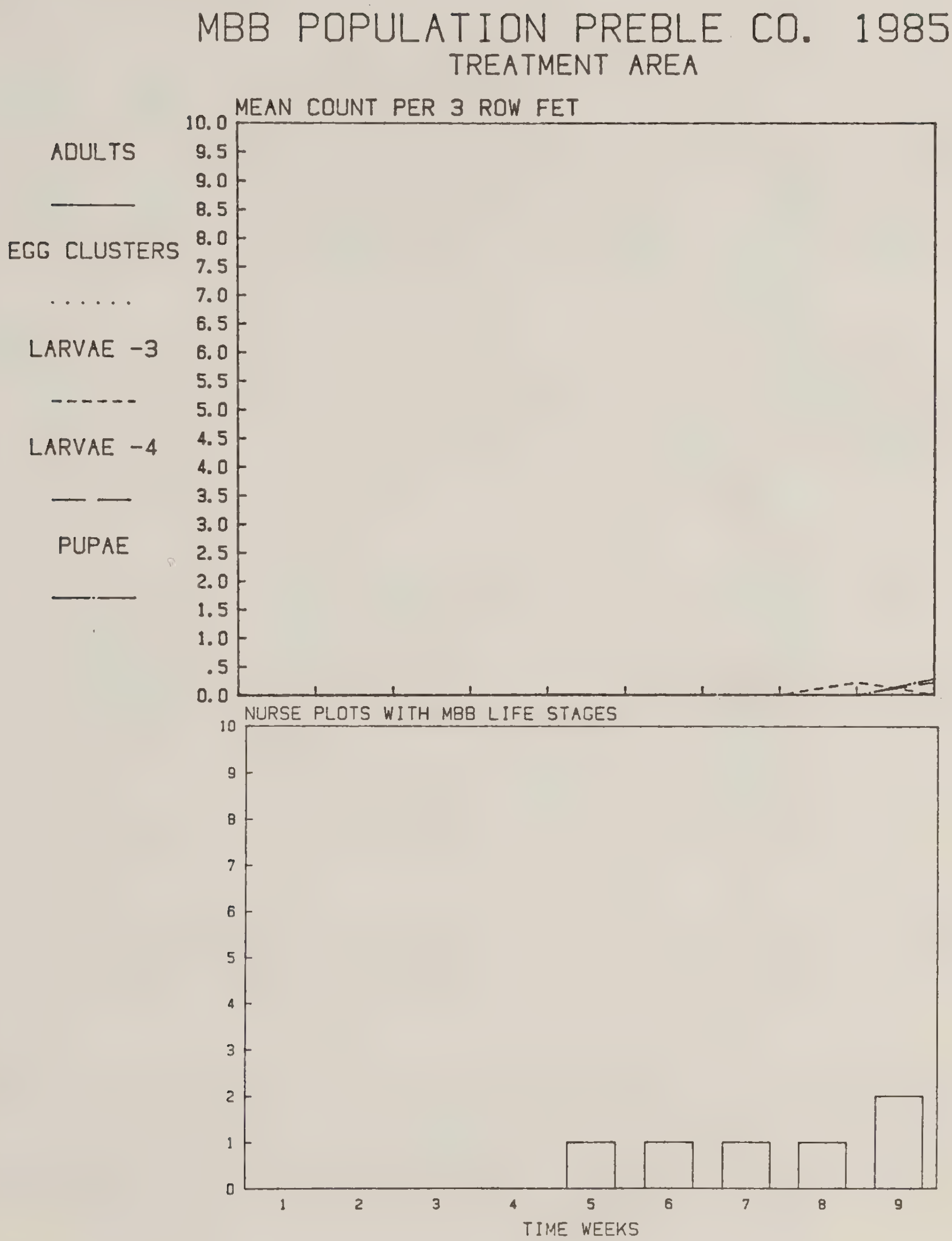
TIME WEEKS

Figure 6.


MBB POPULATION PREBLE CO. 1985
TREATMENT AREA
MEAN COUNT PER 3 ROW FET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
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7.0
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4.5
4.0
3.5
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2.5
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1.5
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NURSE PLOTS WITH MBB LIFE STAGES
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
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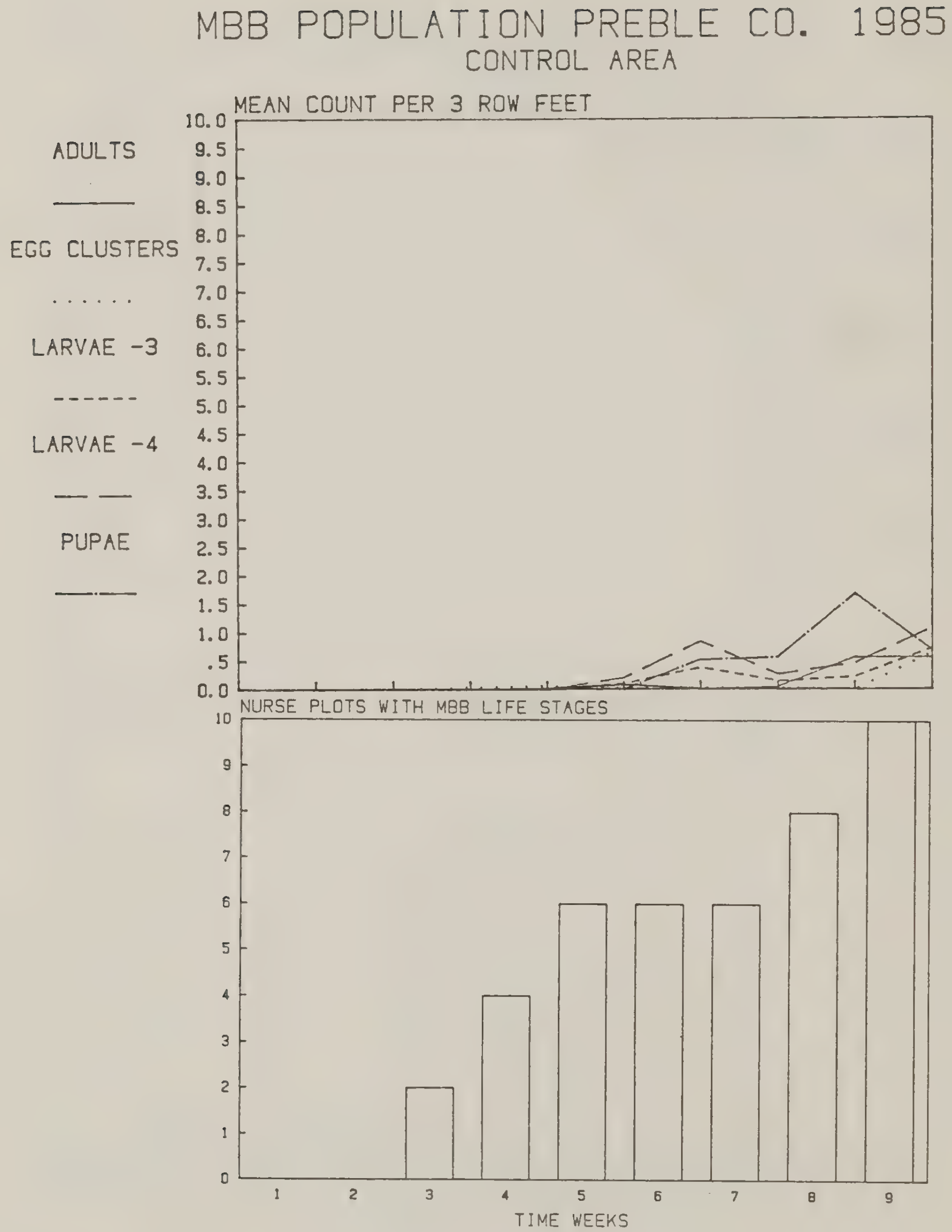
6
7
8
9
TIME WEEKS

Figure 7.


MBB POPULATION PREBLE CO. 1985
CONTROL AREA
MEAN COUNT PER 3 ROW FEET
ADULTS
EGG CLUSTERS
LARVAE -3
LARVAE -4
PUPAE
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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0.0
NURSE PLOTS WITH MBB LIFE STAGES
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
TIME WEEKS

Project Number: JB 4.1.1
Project Title: Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports.
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

Japanese beetle infestations are known to occur at nearly all major airports in the eastern United States and, at times, adult populations are high enough to warrant regulation of airports. Adult beetles are attracted to aircraft and can easily hitchhike to western states and establish new infestations. For example, aircraft inspections in California have resulted in beetle finds on a number of planes that originated from infested airports in the east.

Infestations can be controlled with soil treatments of insecticide. However, insecticides such as chlordane are no longer registered for this use. Oftanol is presently registered but is not as effective as the materials applied in the past. During the past few years, alternative control techniques have been used. Some are aircraft cabin treatments with d-Phenothrin, trap crops, soil liming and pheromone and bait traps.

When trapping beetles at heavily infested airports, a major problem is having the manpower to empty all traps before they become overflowing and no longer effective. Recently, at Dulles International Airport, Ron Addington developed a trap design that eliminates the handling of captured beetles. A tube sock, with the toe end cut off and treated with 5% Sevin dust (Sevin 80S), was attached to the bottom of a standard trap. The bottom of the collection can is cut out so beetles will pass through. As the beetles pass through, they contact the material with mortality resulting. Beetles pass through the trap and fall to the ground, eliminating the need to empty traps. Socks are dusted with Sevin on a weekly basis or more frequently if rain occurred.

Laboratory studies have demonstrated the residual of some insecticides and/or formulations to be greater than that of Sevin 80S. If this is true under field conditions, retreatment might have to be done less frequently during the trapping season.

A field trapping test was conducted on Otis Air National Guard Base with the tube sock trap and 8 contact insecticides (12 formulations). The main objective was to collect trapped beetles and record mortality to determine field residual of the tested formulations. This study was started in June and terminated in early September when beetle populations declined.

An open grassy field, about 50 acres in size, was selected as the test site. Japanese beetle grub population in the area was approximately 15 grubs per square foot. Six parallel lines of traps were established so that traps were 50 feet apart in all directions. The bottoms of the collection cans of Ellisco traps were removed to make an opening for beetles to fall through. The toe end of tube socks (K-Mart) were removed and the ankle end pulled up completely over the perforated collection can. The open top of a cardboard container was attached to the end of each sock. A plastic funnel was attached to the bottom of each sock so that captured beetles were not able to climb back up into the treated sock. A container was placed into the ring top to collect all trapped beetles. In an operational program, all trapped beetles fall out the end of the sock onto the ground. Traps were suspended approximately 3 feet off the ground by a steel rod. Each treatment was replicated 6 times with a treatment trap on each line. After each collection, traps were relocated on the lines.

On June 20, 1985, traps were put out and treated with test insecticides. Dust was applied to the inside of the main trap funnel and outside of sock by a small hand-held duster. Liquid formulations were applied by submerging the entire sock in the insecticide, then pulling it over the collection can. Traps were baited with standard lure and pheromone. Six standard Ellisco traps (unaltered) were included as checks. Each trap was given a code number to identify the various treatments.

Trapped beetles were removed to the laboratory and held for 24 hours at which time mortality readings were made. Beetles were assumed to be dead when no movement occurred after being prodded with a brush. After mortality was recorded, beetles and collection containers were discarded to prevent contamination. During peak flight time (July - mid August), collection containers were placed onto traps for 1 hour for each sample. If left on longer, excessive numbers of beetles were captured.

On July 8, 1985, a second application of test materials was applied. Socks and funnels were treated with dust materials using the same treatment technique as the original application. Liquid materials were applied with a hand-held pump up sprayer.

On August 8, 1985, a series of new traps were treated with Sevin XLR and Malathion. Various parts of the traps were treated in order to pin-point the best location for the insecticide to be applied.

Table 1. Percent mortality of Japanese beetles 24 hours after capture in insecticide treated sock type traps over a period of time.

	Percent mortality days after trap treatment												
Material	1[1/] day	3 days	12 days	16 days	18 days	1[2/] day	7 days	12 days	22 days	30 days	37 days	46 days	60 days
d-Phenothrin 10%A	100	100	0	5	6	28	0	0					
Malathion 5% D	100	100	0	1	41	73	36	2					
Malathion 5% EC	100	100	50	93	45	54	67	63	76	86	79	59	92
Methoxychlor 5% D	100	96	0	1	8	1	5	1					
Methoxychlor 5% EC	67	20	67	73	5	4	7	11					
Orthene 5% D	100	100	0	17	38	73	42	12					
Pounce 5% D	100	100	0	57	3	5	0	10					
Pounce 5% EC	100	91	0	86	20	22	0	41					
Pydrin 5% EC	88	79	33	66	19	4	4	72					
Pyrenone 5% EC	100	100	0	39	0	65	7	51					
Sevin XLR 5% EC	100	94	0	96	88	81	97	96	91	89	97	94	95
Sevin 80S 5% D	100	100	50	65	21	87	68	18	11	1	5	32	42
Ellisco Std.	20	19	0	0	0	1	0	0	0	0	1	2	10

1/ Days after 1st treatment - June 20, 1985.

2/ Days after 2nd treatment - July 8, 1985.

Table 2. Percent mortality of Japanese beetles based on total of all beetles collected throughout testing period.

Material	Totals for test period			Percent mortality
	Alive	Dead	Total	
d-Phenothrin 10% A	1494	115	1609	7
Malathion 5% D	1382	547	1929	28
Malathion 5% EC	1020	4203	5223	80
Methoxychlor 5% D	1990	57	2047	3
Methoxychlor 5% EC	2583	182	2765	7
Orthene 5% D	1079	371	1450	26
Pounce 5% D	2415	230	2545	9
Pounce 5% EC	2703	412	3115	13
Pydrin 5% EC	2803	519	3322	16
Pyrenone 5% EC	1538	428	1966	22
Sevin XLR 5% EC	616	8498	9114	93
Sevin 80S 5% D	7444	1046	8490	12
Ellisco Std.	10179	116	10295	1

Table 3. Percent mortality of Japanese beetles 24 hours after capture in traps that had various sections treated with insecticide.

Material	Section treated	Percent mortality following treatment 1 day	8 days	11 days	16 days	22 days	25 days
Sevin XLR 5% EC	Complete trap	97	97	87	70	89	78
"	Can only	99	73	92	86	92	85
"	Sock only	6	30	32	23	47	49
Malathion 5% EC	Complete trap	91	25	33	66	61	91
"	Sock only	9	7	41	35	44	36
Standard Ellisco	-	1	0	0	0	0	2

Treatments made August 12, 1985.

Table 4. Percent mortality of Japanese beetles based on total of all beetles collected throughout testing period.

Material	Section treated	Alive	Dead	Total	Percent mortality
Sevin XLR 5% EC	Complete trap	132	2055	2187	94
"	Can only	133	1458	1591	92
"	Sock only	3115	895	4010	22
Malathion 5% EC	Complete trap	442	1191	1633	73
"	Sock only	2877	508	3385	15
Standard Ellisco	-	1779	85	1864	5

Table 5. Rainfall amounts that were recorded in the Japanese beetle trap study area.

June		July		August	
Date[1/]	Inches rain	Date	Inches rain	Date	Inches rain
6/23	0.11	7/11	0.17	8/1	0.72
6/24	0.18	7/16	0.59	8/8	1.22
6/25	0.02	7/17	0.52	8/16	0.02
6/26	0.02	7/22	0.87	8/19	0.20
6/27	0.49	7/26	0.89	8/25	1.85
6/28	0.04	7/27	0.07	8/26	3.37
		7/31	0.02	8/30	2.74
Totals	0.86		3.13		11.19
		Average temperature			
1000	58°F		66°F		65°F
1400	66°F		75°F		72°F

1/ Started recording rainfall only after traps were out.

Test results indicate a much longer residual with Sevin XLR 5% than any of the other tested materials. Malathion 5% EC was also effective for a longer period of time than the presently used Sevin 5% dust. Most materials were very effective the first few days and then tapered off rapidly. Sevin XLR continued to be very effective after nearly 2 months.

Results indicate that it is also best to treat the complete trap or a minimum of the can and sock including the inside of the trap funnel.

It is suggested that personnel using Japanese beetle sock type traps start a gradual changeover to Sevin XLR, 5% EC in 1986. The material can be applied with a hand-held pump up sprayer and will eliminate the need for a number of treatments during the trapping period. It may be possible to get by with an original and mid-season treatment only, regardless of weather conditions.

Project Number: CPB 5.1.1
Project Title: Laboratory and Field Tests with *Edovum puttleri* for Controlling *Leptinotarsa decemlineata*
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: O. T. Forrester

Colorado potato beetle (CPB) is the most important insect pest of potatoes on Long Island. It is difficult to control because of its increasing resistance to insecticides. Insecticide application schedules for commercial potato production are, in general, not compatible with the use of biological control organisms. Therefore, any attempt at large scale release and study of *Edovum puttleri* (*Ep*), if unsuccessful, could detract from long range goals of biological control.

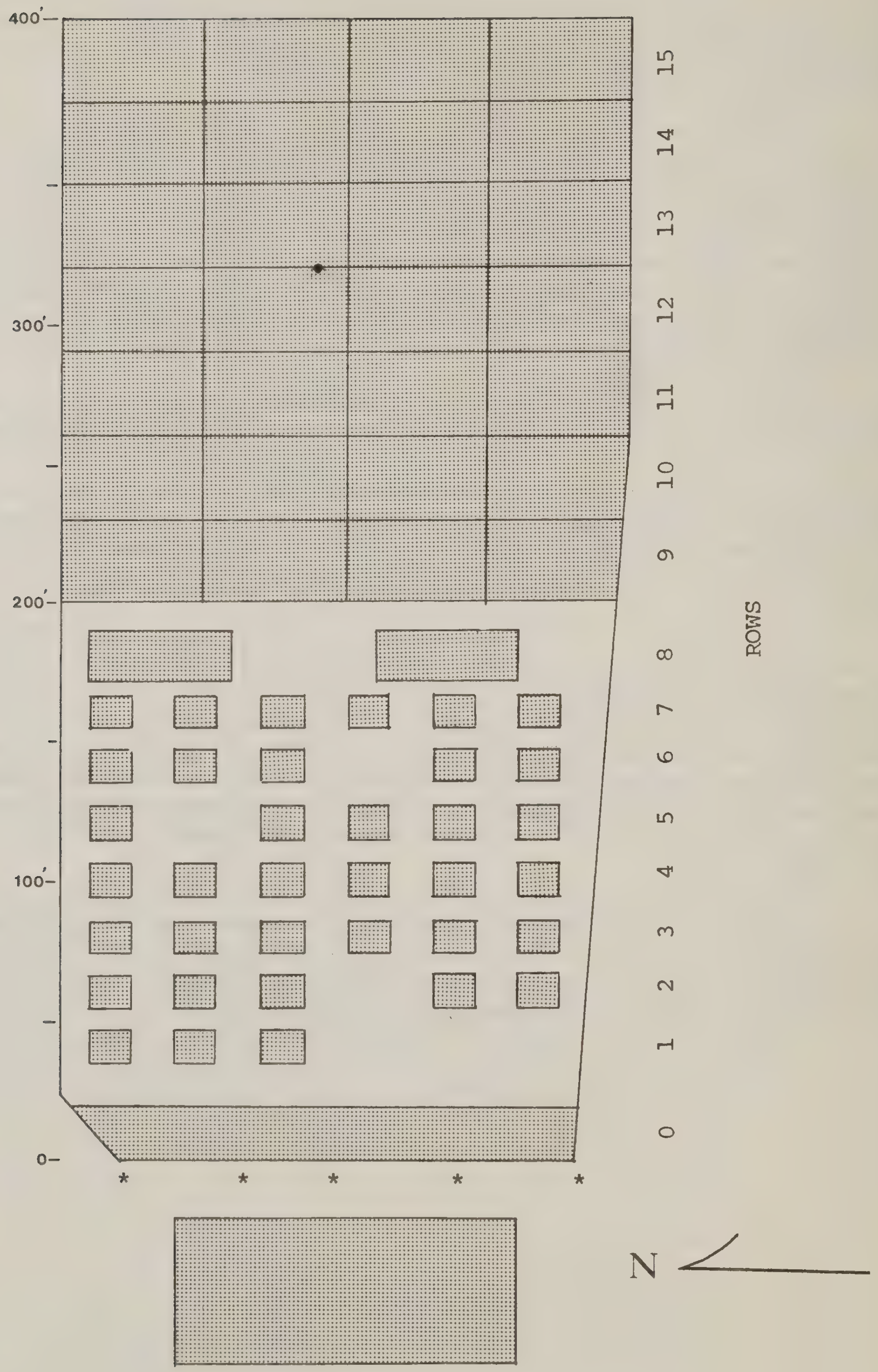
The objective of this project was to establish *Ep* and follow its dispersal without interfering with commercial potato pest control procedures.

This test was conducted on the golden nematode research farm at Farmingdale, New York in conjunction with their ongoing golden nematode trap cropping tests. Normal insect and disease control schedules were followed with (SN 72129 at 0.15 lb. a.i./acre and Dimethane M-45 1-1/2 lb. a.i./acre every 7 days).

Ep was released along the west edge of the 1.6 acre research plot at 1,000 per release on June 19 and 26 (see Figure 1). CPB egg clusters were sampled June 26, July 2, 10, 17, 26 and August 2. A maximum of 6 egg clusters were sampled from each row and held at 25°C until all eggs hatched or *Ep* eclosed. The results of this test are given in Figures 2 through 4. The highest parasitism was observed on July 17 (sample period 4). Parasitism was not observed in rows 3, 5 or 7 nor was it observed beyond 200 feet; sampling was conducted to 250 feet.

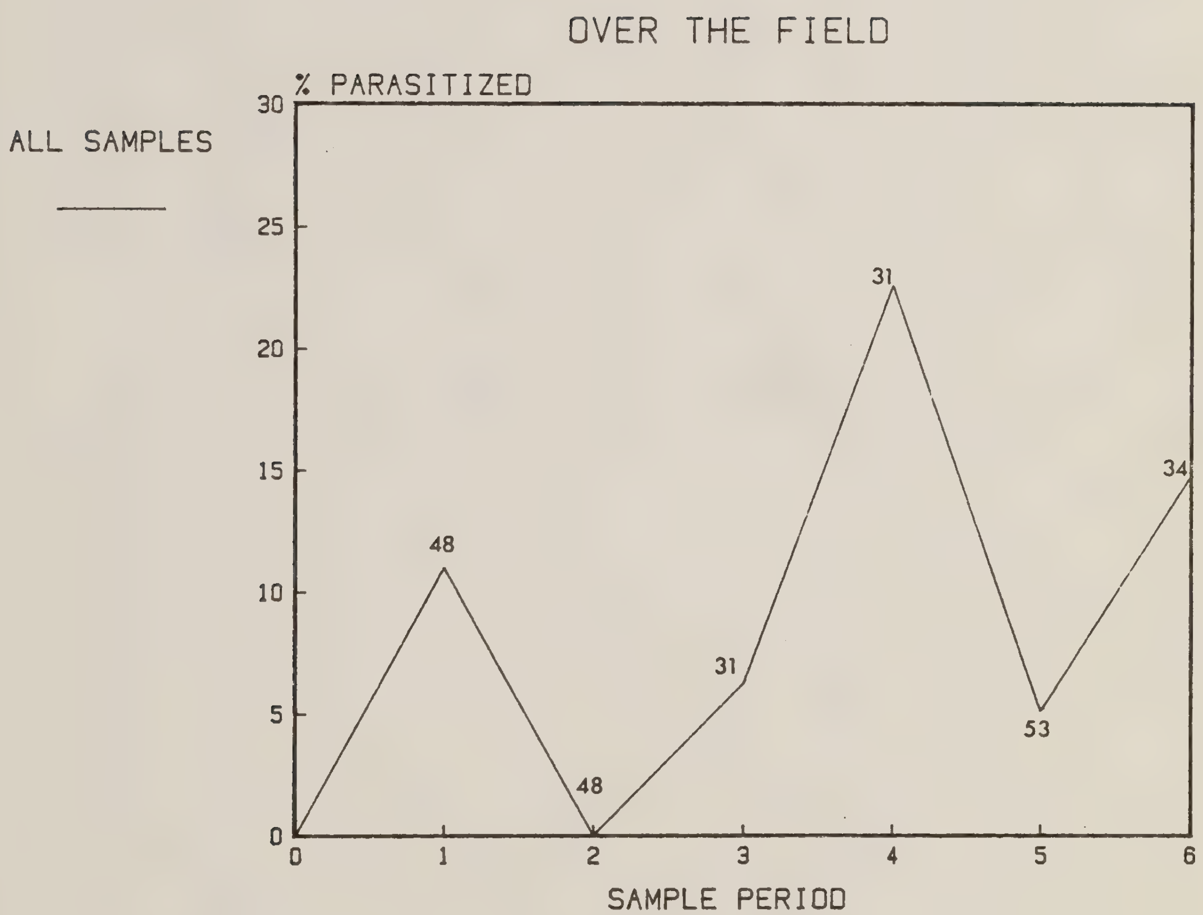
It is possible for *Ep* to establish under commercial control schedules if a material (such as SN72129) is used that is selective to the extent that beneficial organisms are not affected. Adult and/or larval beneficials observed included *Ep*, Coccinellidae, Syrphid flies and mummified aphids.

Figure 1.



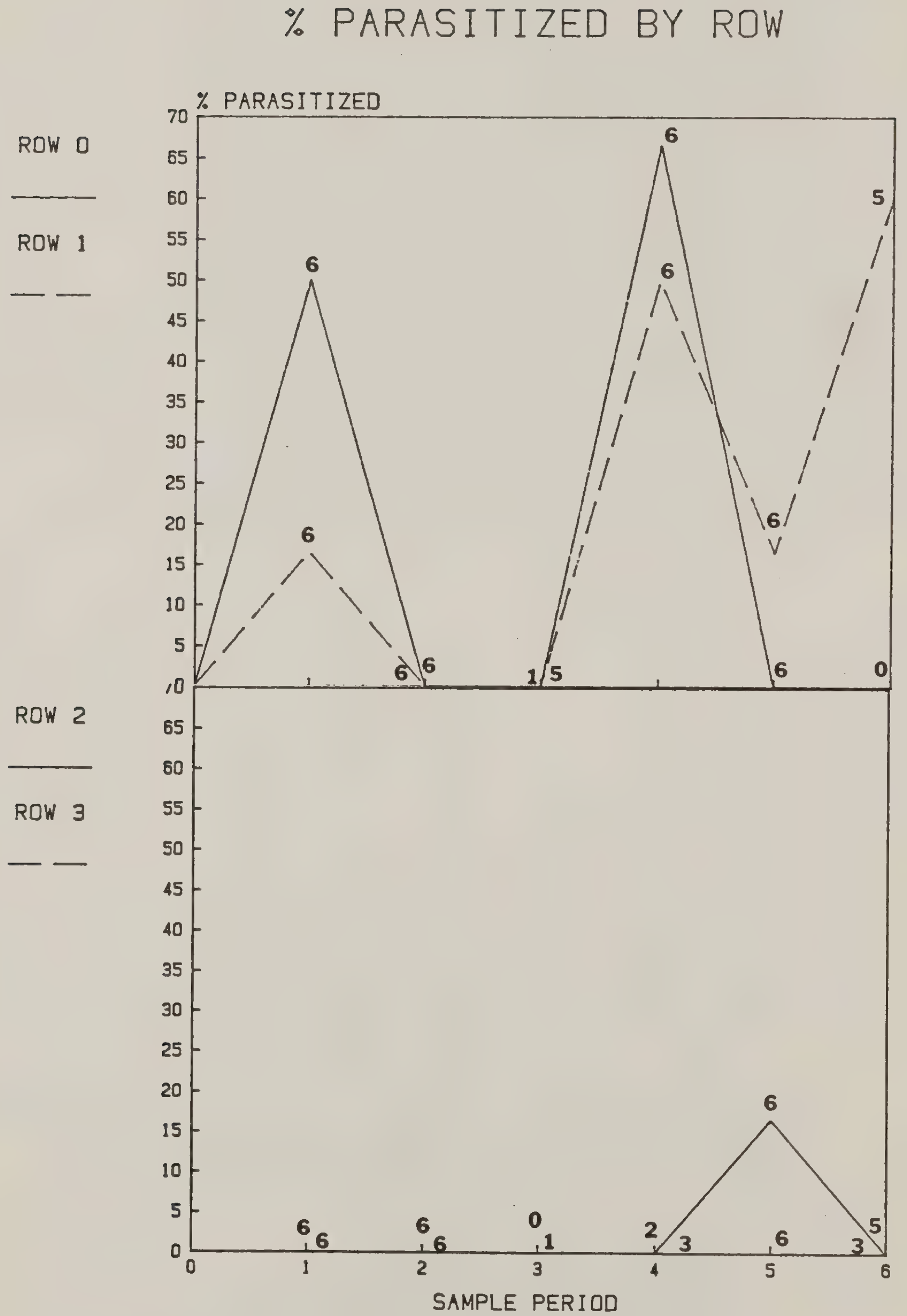
* = Ep release point

Figure 2.



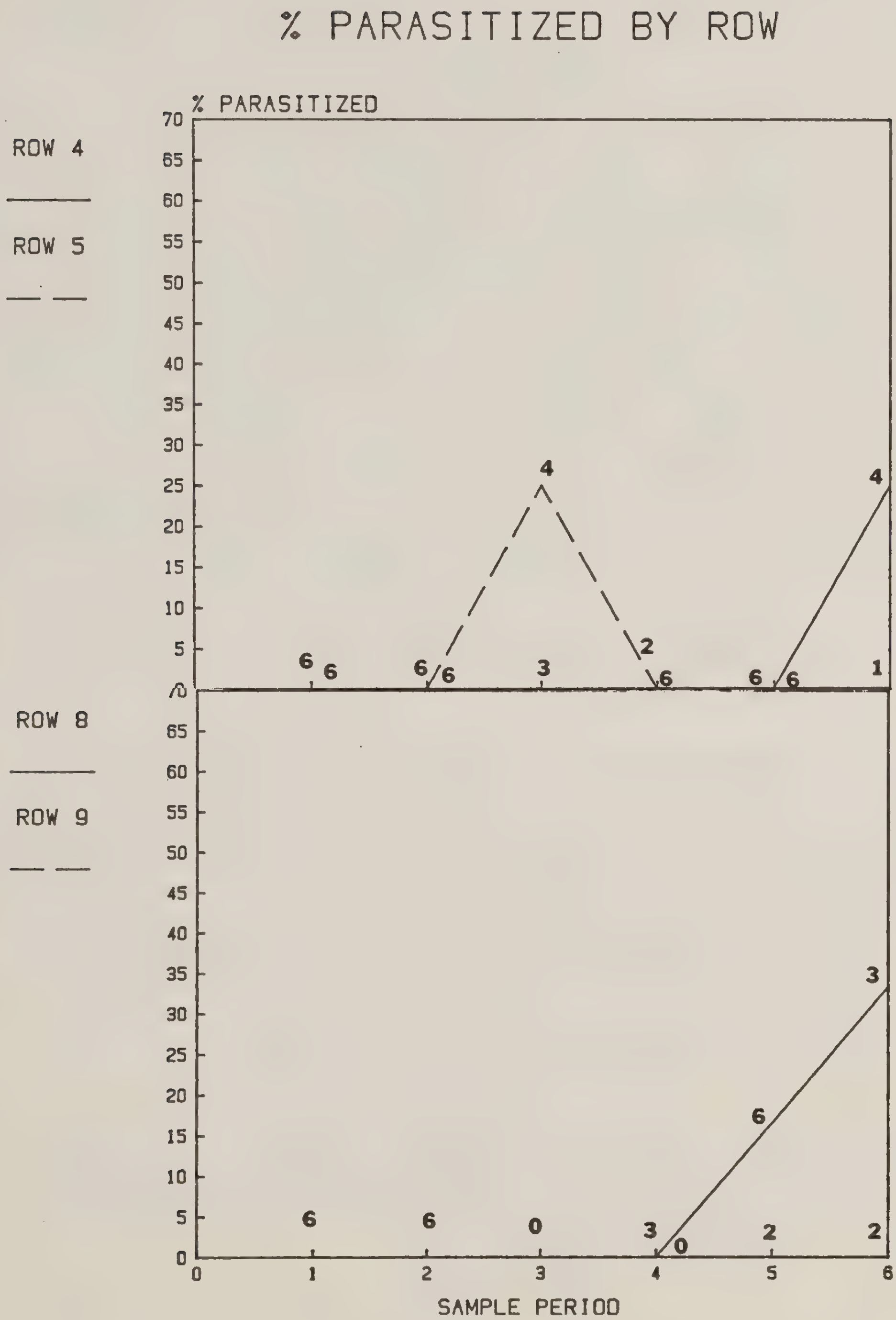
OVER THE FIELD
% PARASITIZED
ALL SAMPLES
30
25
20
15
10
5
0
48
48
31
31
53
34
0
1
2
3
4
5
6
SAMPLE PERIOD

Figure 3.



% PARASITIZED BY ROW
% PARASITIZED
ROW 0
ROW 1
ROW 2
ROW 3
SAMPLE PERIOD

Figure 4.


% PARASITIZED BY ROW
% PARASITIZED
ROW 4
ROW 5
ROW 8
ROW 9
SAMPLE PERIOD

Project Number: CPB 5.1.1
Project Title: Laboratory and Field Tests with Edovum puttleri for Controlling Leptinotarsa decemlineata
Report Period: October 1, 1984 - September 30, 1985
Report Type: Interim
Project Leader: P. C. Kingsley

Field Tests

In cooperation with Robert Bugg, a Research Associate with the New Alchemy Institute, we conducted two experiments in 12 small (5m x 5m) isolated potato plots (var. Katahdin) located at least 100 meters apart on Otis Air National Guard Base. For a more detailed account of the materials and methods and a more thorough discussion of the results of these experiments, please see Robert Bugg's research report to NAI. In the first test, we attempted to enhance *Edovum puttleri* (EP) establishment through the use of nectar-producing plants (several species of Umbelliferae). We knew, through observation in the laboratory (see EP adult diet test below), that a source of carbohydrates was essential to this wasp's survival and that Umbelliferae serve as this source to many Eulophids (R. Bugg, personal observations). Endemic *Leptinotarsa decemlineata* (CPB) populations were absent or very low in these plots, so each was innoculated with 20 unsexed field collected CPB on June 15 and 16 collected. Releases of 100 and 50 mated and pre-exposed female parasites were made in each plot on June 22 and July 3, respectively. As we failed to observe any visitation by EP to flowers, we concluded that EP must be using an alternate source, such as aphid honey-dew, for these sugars.

We designed a second experiment concerning EP release rates in relation to establishment, and possible second year effects on CPB populations. Between CPB generations (late July), 10 of the 12 plots (2 plots were left as unsprayed controls) were sprayed with pyrethrum in an attempt to reduce EP populations to low levels. A third release of EP was then made in 8 plots (2 plots were nearly defoliated and were not used) on July 31 at three different rates (200, 400 and 600 wasps with a 7:1 F:M ratio) and a control of no wasps. Each rate was replicated in two plots. Second generation CPB populations proved very low, although parasitism rates were relatively high. There was no correlation between release rates and parasitism.

The phenologies of CPB life stages, averaged for all 12 plots, are given in Figure 1 and parasitism rates of CPB egg masses in Figure 2. We established EP in 11 of the 12 plots and reared over 1000 egg masses in the laboratory. Even though both tests yielded negative results, we gained valuable experience regarding this host-parasite-plant system.

EP Longevity Test:

The objective of this test was to determine the survival rate of adult EP in the field. Approximately 500 EP (5:1 F:M) were released on July 1 in a small (5m x 5m) isolated potato plot (var. Katahdin). This plot was surrounded by woodland on three sides and a building on the fourth side and was free of endemic CPB populations (potatoes had never been planted in this area). For these reasons, we felt we could successfully monitor parasitism by these wasps in the absence of reproduction, and thereby estimate EP's longevity in the field. Sentinal egg masses were used for this monitoring and consisted of irradiated CPB egg masses glued to a 3.5 cm diameter disk of filter paper. Ten such disks were pinned to the underside of potato leaves, selected at regular intervals, in the field. Sentinals were replaced every 48 hours. The percentages of egg masses and eggs parasitized are given in Figure 3. Even though there was no reproduction in this plot (no native CPB egg masses were ever found), sentinal egg masses were still parasitized 48 days after release. Although this estimate of longevity may seem unreasonable, individual EP females have lived for over 60 days in the laboratory (personal observation). Another possible explanation might be that a native species of nightshade, in the surrounding woodlands, could have harbored a small population of CPB and thereby supported EP reproduction. Beetles were never found on these few plants, however, and sentinal egg masses on potted potato plants, placed approximately 10 meters outside the field edge, were not parasitized. We plan to run a similar test next year in an area without nightshade.

Sentinal Egg mass Test:

In addition to monitoring EP longevity in the plot mentioned above, we simultaneously tested the attractiveness of various sentinal egg masses and the influence of plants fed upon by CPB, on parasitism. On July 2 and 3, in addition to the 10 irradiated sentinals, we placed freshly laid non-irradiated egg masses in the plot. These masses were pinned directly to leaves in an attempt to simulate endemic eggs. Although 20% (4) fewer sentinal egg masses were parasitized, this difference was not statistically significant (Chi-square).

Treatment	No. Egg Masses	Percent Egg Masses Parasitized
Sentinals	20	60
Fresh	20	80

On a second test, we compared egg masses laid on potted plants fed on by CPB with sentinals (egg masses glued to filter paper). A significantly higher number of egg masses on the plants exposed to CPB were parasitized.

Treatment	No. Egg Masses	Percent Egg Masses Parasitized
Sentinals	10	20
Fresh Egg Masses on Potted Plants	33	73

Finally, sentinals on CPB-exposed potted plants and sentinals on field plants (no CPB exposure) were compared. Again, plants exposed to CPB's feeding or frass seemed to infulence parasitism rates.

Treatment	No. Egg Masses	Percent Egg Masses Parasitized
Sentinals	10	40
Sentinals on Potted Plants	5	100

These tests suggest that sentinal egg masses, in native CPB populations, may be of limited value unless they are placed on plants where CPB have been feeding. In a no-choice situation, however, such as the longevity test above, useful information can be collected.

Laboratory Tests

Edovum puttleri Adult Diet Tests:

These tests were conducted by Sharon Rainsberry, with the objective of determining the effects of carbohydrate and protein sources on the fecundity and longevity of adult EP in the laboratory.

Fecundity Test:

Female EP were reared, individually, under five diet regimes; honey, honey with bee pollen, a mixture of sugars to simulate nectar*, pollen alone, and water as a control. A CPB egg mass, with at least 20 eggs, was offered to each female every 48 hours. The test was terminated after 32 days.

Diet Treatment	No. of Females	Mean (SE) No. of CPB Eggs Parasitized Per Day/Female
Honey	10	4.6 (0.61) a
Honey and Pollen	10	3.5 (0.78) a
Nectar*	10	3.7 (0.93) a
Pollen	10	1.5 (0.38) b
Control (Water)	10	0.5 (0.24) b

* 52.6% Fructose, 45.7% Destrose, 1.7% Sucrose by dry weight; from Erickson, et al. 1979. J. Amer. Soc. Hort. Sci. 104(5):635-638.

Diets with a carbohydrate source supported higher rates of egg production than those without. Pollen alone did not significantly affect fecundity.

<u>Longevity Test:</u>

Female EP were again reared individually with five diet regimes as above, with an additional treatment of honey without eggs to test the effect of egg feeding on survival. The first of two trials was terminated after 34 days and the second after 26 days.

Diet Treatment	No. of Females	Mean (SE) Longevity
Honey	19	26.9 (1.50) a
Honey - Eggs	19	23.6 (2.40) a
Honey and Pollen - Eggs	17	22.8 (2.40) a
Nectar - Eggs	16	14.2 (1.74) b
Pollen - Eggs	18	6.2 (0.81) c
Control (Water)	17	5.5 (0.52) c

As with fecundity, longevity was significantly higher when females were given a carbohydrate source. The significant reduction in the nectar treatment may have been due to an "artificial" mortality caused by the stickyness of the nectar solution. The absence of eggs from the honey treatment did not influence survival. In a separate test, the presence or absence of eggs was tested without a sugar source with the same negative results.

Diet Treatment	No. of Females	Mean (SE) Longevity
Water + Eggs	10	3.1 (0.53) a
Water, No Eggs	10	2.3 (0.37) a

CPB Egg Storage Tests:

In this test, conducted to a large extent by Tracy Ellis, we studied the feasibility of using irradiated CPB eggs as hosts for rearing EP. The use of irradiated eggs in a rearing operation would have two major advantages. First, since these eggs would be dead, parasitized egg masses would not have to be screened to eliminate emerging cannibalistic host larvae from unparasitized eggs. Secondly, it might be possible to store irradiated eggs longer than is now possible for non-irradiated eggs. With this last advantage in mind, we tested several parameters: 1) acceptability, or the percentage of egg masses parasitized, 2) parasitism, or the percentage of eggs producing F_1 adults, 3) the sex ratio of these adults, and 4) the fecundity of the F_1 females. What follows are some preliminary results.

Freshly laid CPB eggs were irradiated with 20 Krads, using a Cobalt 60 source, and stored at 5^{o}C for six weeks. Two additional devitalization treatments were also tested, microwaved and frozen. Stored eggs, as well as freshly laid eggs as a control, were submitted to EP in petri dishes for 24 hours.

Treatment	Percent of Egg Masses Parasitized	Mean (SE) Percent Parasitism/Egg Mass	Mean (SE) Percent Females/Egg Mass
Control	61.5 (16/26) a	60.7 (0.06) a	70.6 (0.06) a
Cobalt 6 Weeks	61.5 (24/39) a	26.9 (0.03) b	76.7 (0.06) a
Microwave 6 Weeks	18.8 (9/48) b	24.0 (0.06) b	75.8 (0.18) a
Frozen 6 Weeks	10.0 (4/40) b	20.8 (0.04) b	62.7 (0.10) a

A Chi-square test indicated no significant difference between the acceptability of fresh egg masses and irradiated, stored egg masses. Egg masses killed by microwave or freezing, and subsequently stored 6 weeks were significantly less acceptable. Irradiated egg masses, stored for 6 weeks only resulted in approximately half the number of wasps as the control eggs. This result could be due to either of two factors: fewer stored eggs were parasitized, or there was a higher mortality of parasites in stored eggs.

A second test, using freshly irradiated and 3 week old irradiated eggs, yielded similar results.

Treatment	Percent of Egg Masses Parasitized	Mean (SE) Percent Parasitism/Egg Mass
Control	98 (90/92)	53.7 (0.02) a
Fresh, Irradiated	97 (74/76)	51.0 (0.03) a
3 Week, Irradiated	96 (80/83)	38.5 (0.02) b

Stored eggs yielded fewer wasps than live eggs or unstored, irradiated eggs.

F_1 females, resulting from the control and irradiated treatments in the first storage test, were reared individually and supplied with egg masses every 48 hours to measure fecundity.

Treatment	Females	Percent of Females Laying Eggs	Mean (SE) Number of Eggs/Female/Day*
Control	13	100	5.8 (0.58) a
6 Wk. Cobalt	7	85.7	6.4 (1.15) a

There was no significant difference between treatments. Experiments are continuing to further investigate the storage of host eggs for EP production.

EP Geotropism Test:

This summer, while inspecting CPB egg masses collected in the field and reared for the determination of parasitism rates, we noticed that a significant number of EP were dying as adults while still in the host egg. It seemed clear that one reason for this mortality was their orientation inside the egg at the time of pupation. That is, those individuals pupating with their head towards the leaf side of the egg had a difficult time exiting from the host egg. Since in the field, the large majority of eggs are laid on the bottom of the leaf surface, we tested the hypothesis that this mortality might arise from the haphazard orientation of egg masses when held in the laboratory. To test for such a geotropic effect, thirty irradiated egg masses were exposed to 200-300 EP adults for 24 hours. Egg masses were then glued to the top of individual rearing containers. Half were reared upside down, or with the eggs above the leaf, and the other half right side up, or with the eggs in the normal, below-leaf orientation. The treatments were then repeated the next day, resulting in a total of 60 egg masses. All eggs were placed in total darkness to avoid effects of light on the parasitoid's orientation.

Treatment	Total Eggs	Overall Parasitism	Mortality*	Wasp Orientation	
				Up	Down
Eggs Up	749	48.5%	27.8%	3.9%	23.14%
Egg Down (Normal)	702	48.2%	4.1%	0	4.1%

* Adult mortality, that is, adult wasps dead inside the host egg.

A significantly higher mortality occurred among emerging adults in the "eggs-up" treatment. Of these, the large majority of wasps (83%) died with their heads orientated down (towards the leaf).

We are presently looking at ways to alleviate this problem in a rearing operation. One obvious method would be to deglutinize the eggs after they had been parasitized, thus allowing the emerging adult to exit either end.

Figure 1. Seasonal phenology of CPB in 12 small potato plots on Otis ANGB, 1985.

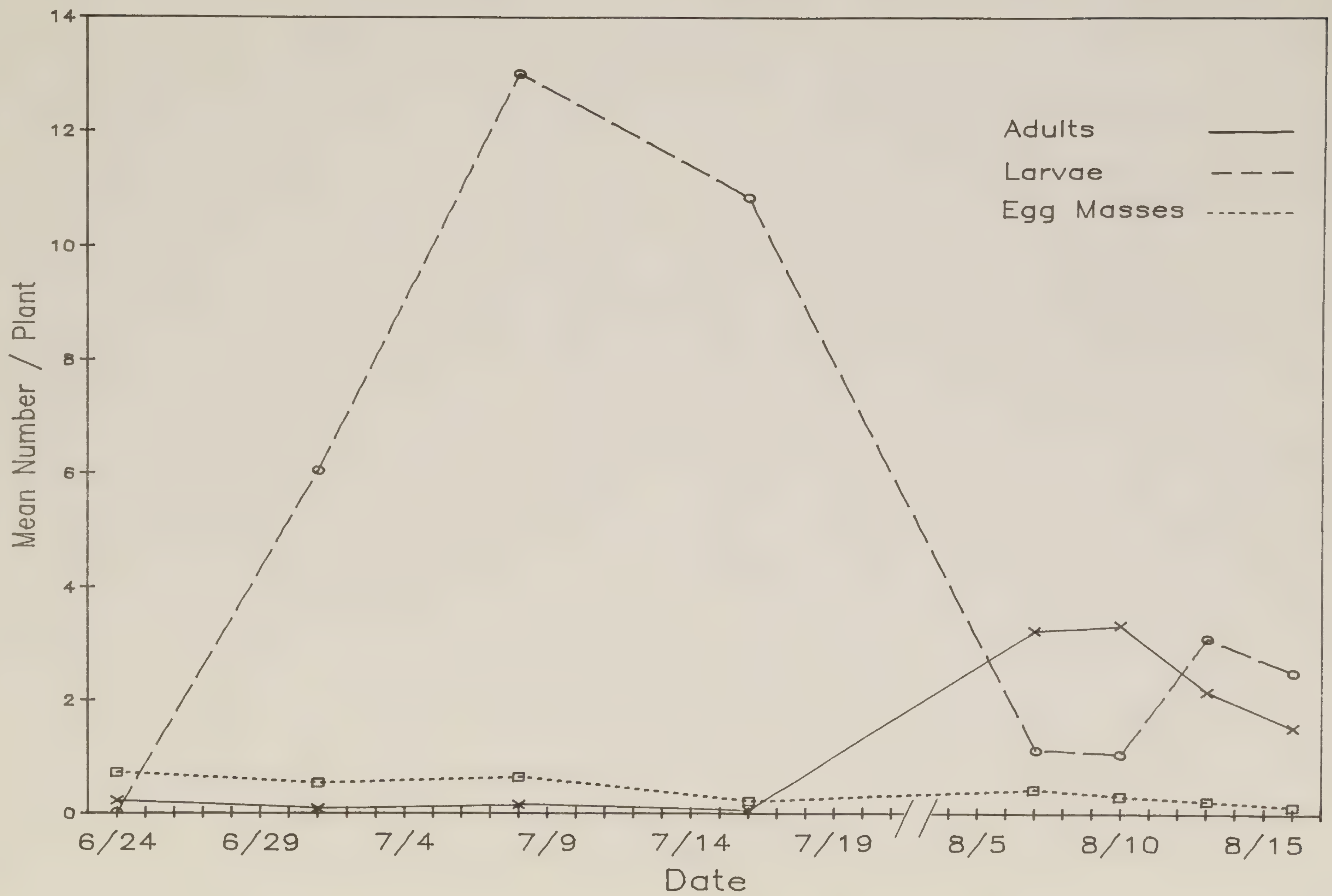


Figure 2. Overall percentage of CPB egg masses parasitized by *Edovum puttleri* in 12 small potato plots on Otis ANGB, 1985. Arrows indicate parasite releases.

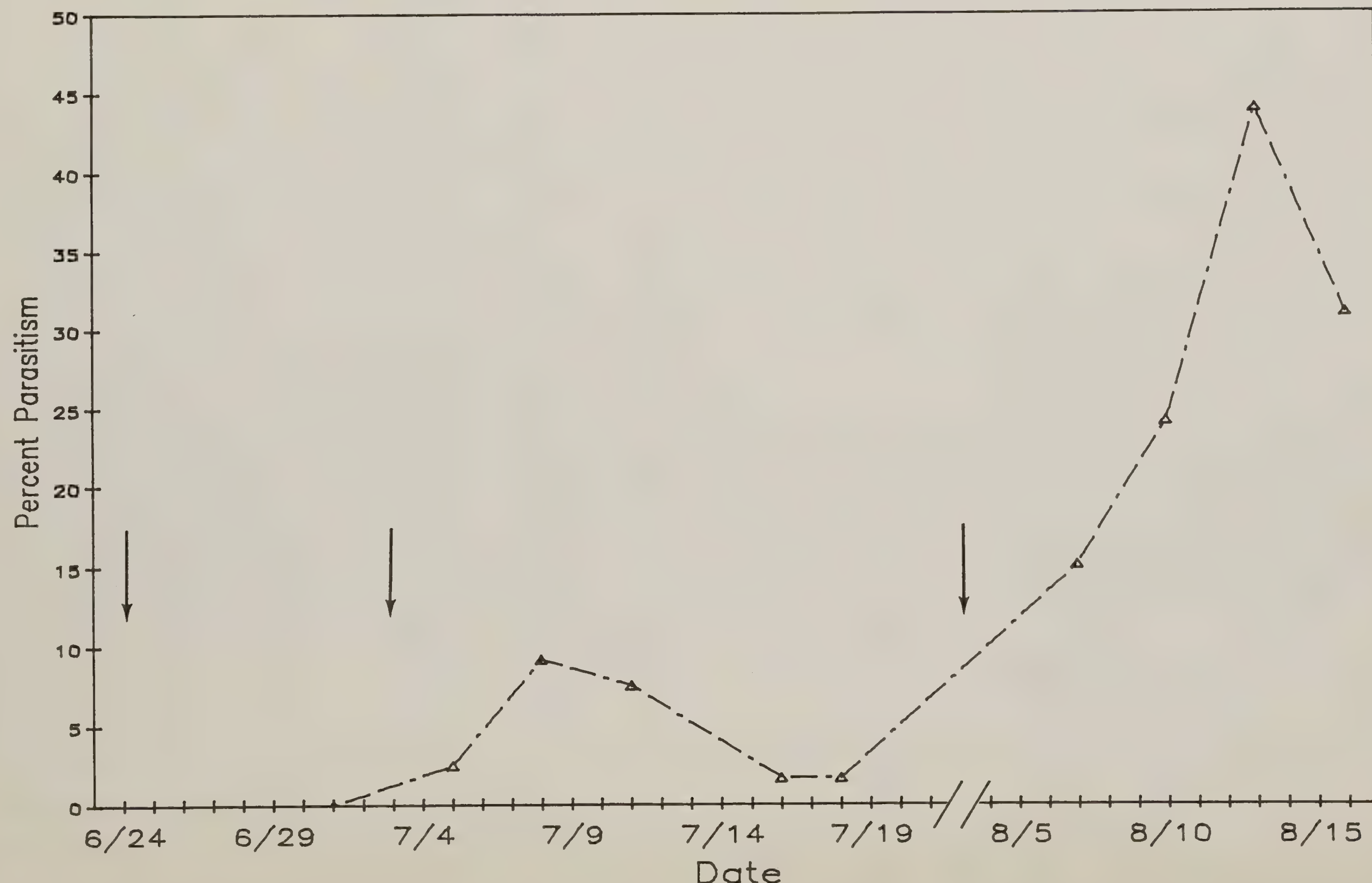
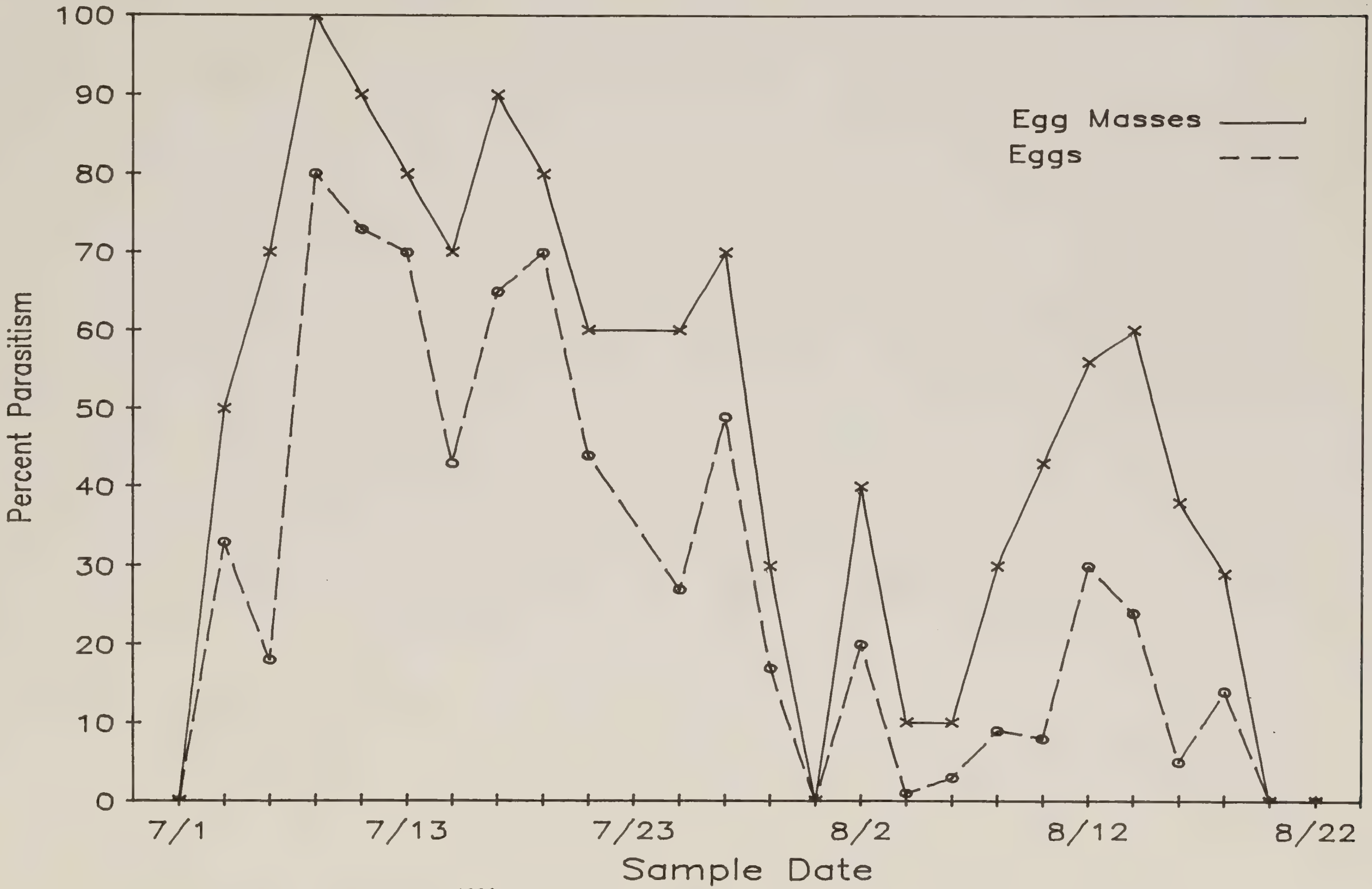


Figure 3. Percentage of ten sentinal CPB egg masses, and the total number of eggs, parasitized by *Edovum puttleri* released on 7/1 in potatoes on Otis ANGB, MA (1985).



* U.S. GOVERNMENT PRINTING OFFICE:1986- 6 0 1 791 30038